EXHIBIT I

Risk Analysis, Vol. 33, No. 12, 2013 DOI: 10.1111/risa.12070

Drinking Water as a Proportion of Total Human Exposure to Volatile *N*-Nitrosamines

Steve E. Hrudey,[1,*] Richard J. Bull,[2] Joseph A. Cotruvo,[3] Greg Paoli,[4] and Margaret Wilson [4]

Some volatile *N*-nitrosamines, primarily *N*-nitrosodimethylamine (NDMA), are recognized as products of drinking water treatment at ng/L levels and as known carcinogens. The U.S. EPA has identified the *N*-nitrosamines as contaminants being considered for regulation as a group under the Safe Drinking Water Act. Nitrosamines are common dietary components, and a major database (over 18,000 drinking water samples) has recently been created under the Unregulated Contaminant Monitoring Rule. A Monte Carlo modeling analysis in 2007 found that drinking water contributed less than 2.8% of ingested NDMA and less than 0.02% of total NDMA exposure when estimated endogenous formation was considered. Our analysis, based upon human blood concentrations, indicates that endogenous NDMA production is larger than expected. The blood-based estimates are within the range that would be calculated from estimates based on daily urinary NDMA excretion and an estimate based on methylated guanine in DNA of lymphocytes from human volunteers. Our analysis of ingested NDMA from food and water based on Monte Carlo modeling with more complete data input shows that drinking water contributes a mean proportion of the lifetime average daily NDMA dose ranging from between 0.0002% and 0.001% for surface water systems using free chlorine or between 0.001% and 0.01% for surface water systems using chloramines. The proportions of average daily dose are higher for infants (zero to six months) than other age cohorts, with the highest mean up to 0.09% (upper 95th percentile of 0.3%).

KEY WORDS: Comparative exposure assessment; dietary intake; endogenous formation; NDMA

1. INTRODUCTION

Volatile *N*-nitrosamines are a class of chemical contaminants that exhibit unambiguous, genotoxic carcinogenicity in rodent bioassays.[1,2] The International Agency for Research on Cancer classified them as probable human carcinogens[3] as does the U.S. Environmental Protection Agency (USEPA).[4]

[1]Analytical & Environmental Toxicology, Department of Laboratory Medicine & Pathology, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, Alberta, Canada.
[2]MoBull Consulting, Richland, WA, USA.
[3]Joseph Cotruvo & Associates LLC, Washington, DC, USA.
[4]Risk Sciences International, Ottawa, Ontario, Canada.
*Address correspondence to Steve E. Hrudey, 10-102 Clinical Sciences Building, University of Alberta, Edmonton, Alberta, Canada T6G 2C3; steve.hrudey@ualberta.ca.

Volatile *N*-nitrosamines have attracted international health risk management attention regarding human exposure through food and occupational activities.

N-nitrosodimethylamine (NDMA) was discovered as a drinking water disinfection by-product (DBP) in Oshweken, Ontario, Canada in 1989[5] and the Ontario Ministry of Environment developed a drinking water standard of 9 ng/L for NDMA in 1992.[6] The California Department of Public Health developed a notification level of 10 ng/L for NDMA in 1998.[7] Since then, the World Health Organization (WHO),[8] the Australian National Health and Medical Research Council (NHMRC),[9] and Health Canada (HC)[10] have adopted drinking water guidelines for NDMA of 100 ng/L (WHO and NHMRC) and 40 ng/L (HC), respectively.

In March 2010, the U.S. EPA began a regulatory determination process for volatile *N*-nitrosamines in drinking water as a contaminant group being considered for regulation. Previously, five *N*-nitrosamines were listed on the third Candidate Contaminant List (CCL-3): NDMA, *N*-nitrosodiethylamine (NDEA), *N*-nitrosodipropylamine (NDPA), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosodiphenylamine (NDPhA). For the second Unregulated Contaminant Monitoring Rule (UCMR2) six volatile *N*-nitrosamines (NDMA, NDEA, NDPA, NPYR, NMEA, and NDBA (*N*-nitrosodibutylamine)) were included for assessment of their occurrence in U.S. drinking water systems and preliminary results for over 18,000 water samples are now available for assessment.[11] NDMA is by far the most commonly detected nitrosamine in drinking waters, usually at less than 10 ng/L when it occurs, with detections above the minimum reporting level (MRL) of 2 ng/L in about 10% of treated water samples and about 27% of predominantly chloraminated drinking water samples.

2. LITERATURE REVIEW

2.1. Formation of Nitrosamines in Drinking Water

Formation of NDMA and other volatile *N*-nitrosamines in drinking water treatment is complex and involves at least the following factors:

- They are primarily formed during disinfection with chloramines.[12,13]
- Even a brief contact time with free chlorine prior to formation of chloramines will substantially reduce NDMA formation.[14]
- Formation of NDMA is slow, suggesting that long residence time distribution system samples should be higher than water plant samples for chloraminated systems.[15]
- Reactions with nitrogenous precursors associated with wastewater-impacted raw water, notably secondary amines, i.e., dimethylamine for NDMA, diethylamine for NDEA, ethylmethylamine for NEMA, and diphenylamine for NDPhA, are important.[16–18]
- Amine-containing cationic polymers used as coagulation aids (e.g., polydiallyldimethyl ammonium chloride or poly DADMAC) can be NDMA precursors, particularly at high coagulant aid dosage,[19] that react with both chlorine[20] and chloramines[21,22] to yield NDMA.
- Reactions with ion-exchange resins having quaternary amine functional groups can yield NDMA.[19]
- Reactions with ozone (O_3) and chlorine dioxide (ClO_2) can produce NDMA under specific circumstances but these agents may also reduce NDMA formation when used as pre-oxidants that can destroy NDMA precursors.[19,23–27]
- Nitrosamines undergo decomposition by ultraviolet light (at doses higher than typically used for disinfection) and other processes.[28,29]

Broad generalizations will not likely be valid for solving a nitrosamine problem at any individual plant. Site-specific investigations must be pursued.[30] Use of chloramines versus free chlorine seems to be the most consistent discriminator for estimating likely presence and concentration ranges of NDMA in treated drinking water.

2.2. Individual Monitoring Studies on the Occurrence of Nitrosamines in Drinking Water

Detailed information on disinfection practices is often missing from the reported surveys, making it difficult to perform an accurate analysis of the full-scale differences between chlorination and chloramination. Many data sources fail to distinguish between use of free chlorine as primary (main) disinfectant with chloramination for secondary (distribution system) disinfection from use of chloramination as primary disinfectant. Because of the limited effectiveness of chloramination as primary disinfectant, in the absence of explicit information, we have assumed that the majority of chloramination applications are for secondary disinfection. Nevertheless, the important aspect of free chlorine contact time and its effect on NDMA formation[14] often cannot be judged because free chlorine contact time is generally not specified.

Table I provides a summary of major published surveys for NDMA, including data from Alberta and Ontario, Canada, California and water treatment plants across North America, Japan, Scotland, England, and Wales. These data provide some consistent findings despite the diversity of survey locations. Median levels of NDMA across all samples were below the MRL, often less than 1 ng/L, for both free chlorine and chloramine plants, i.e., NDMA was not detectable. For free chlorine, both plant effluent

Table I. Summary of Major Studies Surveying Water Treatment Plants for NDMA

Region	Plants (Samples)	Water Source	Disinfection Type	Max (ng/L)	Min (ng/L)	Median[a] (ng/L)	Percent Above MRL (ng/L)
North America 2001–2002	12(48)	SW & GW	**Chloramines**				MRL = 0.6 – 1.0
Barrett *et al.* 2003[34]	12(67)		Plant effluent	6.6	<0.6	<1[b]	33%
Valentine *et al.* 2005[35]			Distribution	21.6	<0.6	<1[b]	75%
21 WTP			**Free Chlorine**				MRL = 0.6 –1.0
	9(30)		Plant effluent	30	<0.6	~2[b]	45%
	9(30)		Distribution	24	<0.6	<1[b]	44%
Ontario, Canada 1994–2002		Not specified	**Chloramines**				MRL =1.0
Ontario MOE as cited in	(277)		Plant effluent	65	<1	1.3	60%
Charrois *et al.* 2007[36]	(76)		Distribution	18	<1	2.2	79%
179 WTP			**Free Chlorine**				MRL =1.0
	(1,429)		Plant effluent	40	<1	<1	42%
	(282)		Distribution	66	<1	<1	35%
California, USA 2002		Not specified	**Chloramines**				MRL = 1.0
CDPH as cited in	(31)		Plant effluent	18.3	<1	1.8	68%
Charrois *et al.* 2007[36]	(34)		Distribution	15.8	<1	1.8	79%
32 WTP			**Free Chlorine**				MRL = 1.0
	(11)		Plant effluent	3.3	<1	<1	27%
	(12)		Distribution	2.5	<1	<1	33%
			Ozone & Chlorine				MRL =1.0
	5(10)		Plant effluent	3.9	<1	<1	50%
	5(10)		Distribution	6.8	<1	<1	30%
Alberta, Canada 2004		SW	**Chloramines**				MRL = 5.0
Charrois *et al.* 2007[36]	8(11)		Distribution	100	<5	<5	45%
20 WTP		SW (1 GW)	**Free Chlorine**				
	8(8)	GW (high NH_3)	Distribution	12	<5	<5	12%
			Free Chlorine				
	4(4)		Distribution	<5	<5	<5	0%
U.S.A.			**Chloramines**				MRL = 2
2006–2007	20	SW	Plant effluent	20	<2	3.3	40%
Mitch *et al.* 2009[37]			**Free Chlorine**				
	7		Plant effluent	<2	<2	<2	0%
Scotland 2008		SW	**Chloramines**				MRL = 1.0
Goslan *et al.* 2009[38]	4		Distribution	26	<1.0	<1.0	not stated
			Plant effluent	13	<1.0	<1.0	not stated
			Free Chlorine				
	3		Plant effluent	<1.0	<1.0	<1.0	0%
England & Wales 2006, 2007		SW & GW	**Chloramines**				MRL = 0.9
Dillon *et al.* 2008[39]	10(16)		Plant effluent	5.7	<0.9	<0.9	31%
		SW & GW	**Free Chlorine**				
	36(64)		Plant effluent	2.8	<0.9	<0.9	14%
Japan 2007, 2008			**Free Chlorine**				
Asami *et al.* 2009[40]			(except 1 WTP)[d]				
# of WTP not specified	(28)	SW[c]	Summer				MRL =1.0
			Plant effluent	2.2	<1	<1	33%
	(31)		Winter Plant				
			Effluent	10	<1	<1	18%

[a]Median of all samples, detected or nondetected.
[b]Estimated from published box and whisker plot, exact value not reported.
[c]Water source was not explicitly described in this article, but the nature of the discussion implies surface waters.
[d]Only one WTP used chloramines and no NDMA was detected in any samples.

and distribution samples were mainly categorized as NDMA not detectable (from 0% to 45% and from 0% to 44% above the MRL, respectively). For chloramine plant effluents, NDMA concentrations were often not detectable (31–68% were above the MRL). Notably, distribution samples for chloramine plants showed more frequent NDMA detection (from 45% to 79% were above the MRL), indicating that chloramines continued to form NDMA during distribution contact time. These findings are generally consistent with the research on causal factors for NDMA formation, most evidently with the greater prevalence of NDMA detection in chloraminated distribution systems. However, some high NDMA levels were reported in free chlorine systems, but there was usually some explanation associated with factors such as use of ion exchange resins. There are substantially fewer data concerning volatile *N*-nitrosamines other than NDMA in drinking water. Charrois *et al.*[31] were the first to report detection of NPYR and *N*-nitrosomorpholine (NMOR) in drinking water. *N*-nitrosamines, other than NDMA, had been reported in wastewater,[32] both before and after chlorination, specifically, NPYR, *N*-nitrosopiperidine (NPIP), and NDEA. Zhao *et al.*[33] reported NDEA, NMOR, and NDPhA in disinfected drinking water.

The most extensive database for *N*-nitrosamines in drinking water is that provided by the UCMR2.[11] Table II summarizes the preliminary results from UCMR2 for the six *N*-nitrosamines. NDMA was the only *N*-nitrosamine that occurred with sufficient concentration and frequency to allow consideration of overall population exposure from drinking water.

Table III provides a summary of the data for NDMA, categorized by source water type and disinfection process for chlorine or chloramines based on UCMR2. This summary confirms that NDMA is a much greater issue for systems using chloramination. Unfortunately, the questionnaire provided with the UCMR2 survey allowed a large number of systems to remain unidentified regarding disinfection process. Among source categories for surface waters, mixed surface and groundwater under the influence of surface water had the highest frequency of NDMA detection above the MRL. The "not identified (NI)" grouping is closer to the chloramine category than to free chlorine, regarding NDMA occurrence, but no further interpretation is possible. Distribution system samples with long residence times have higher NDMA concentrations than plant effluents. Therefore, distribution system values were used for our NDMA exposure assessment modeling.

WHO[8] and HC[10] relied on an overview of dietary and other sources of exposure to NDMA to conclude that drinking water is only a minor source, likely less than 10%, of total exogenous exposure. Fristachi and Rice[41] performed a Monte Carlo analysis to assess the proportion of drinking water to total human exposure and concluded that at a mean NDMA concentration of 2 ng/L, drinking water contributes less than 3% of total external exposure, but if endogenous formation is considered, the drinking water contribution of *N*-nitrosamine (as NDMA) drops to less than 0.02%. They acknowledged considerable uncertainty in the evidence for their estimation of endogenous NDMA formation and their analysis was done without the benefit of the UCMR2 database on *N*-nitrosamines in drinking water or a rigorous analysis of endogenous NDMA formation using a pharmacokinetic approach.

2.3. Endogenous Formation of Volatile *N*-Nitrosamines

This article addresses the contribution of sources of volatile *N*-nitrosamines by analyzing more recent published evidence on exogenous sources of daily human exposure (diet, water) and more detailed and comprehensive analysis of endogenous formation of volatile *N*-nitrosamines than previously undertaken, for the purpose of determining what proportion can reasonably be attributed to drinking water. The following section describes the research underlying the estimate of endogenous NDMA formation on which our model is based. A key issue is the extent to which infants may be at greater risk because of variations in their exogenous exposure or whether there are reasons to suspect that their physiology would lead to greater or lesser endogenous exposure to volatile *N*-nitrosamines.

Volatile *N*-nitrosamines may be formed in the body by acid-catalyzed nitrosation (particularly in the acid stomach) and biologically-catalyzed nitrosation, including systemic nitrosation. Systemic nitrosation depends on NO synthases (neuronal, endothelial, or inducible), that generate the nitrosating agent nitric oxide from arginine in a variety of tissues.

There are several approaches for estimating endogenous formation of volatile *N*-nitrosamines. Krul *et al.*[42] utilized an *in vitro* model system to estimate the amount of NDMA that could be formed by the acid-catalyzed mechanism in the gastrointestinal tract. Other approaches are possible based

Table II. Preliminary Results for Nitrosamine Monitoring Data UCMR2[11]

N-Nitrosamine	MRL (ng/L)	Number of Analyses	Number of Detections (% of Analyses)	Number of Systems with Analyses	Number of Systems with Detects (% of Systems)	Maximum Detected Concentration ng/L
NDEA	5	18,038	46 (0.26%)	1,198	26 (2.2%)	100
NDMA	2	18,040	1,841 (10.2%)	1,198	324 (27%)	630
NDBA	4	18,043	9 (0.05%)	1,198	5 (0.4%)	21
NDPA	7	18,049	0 (0%)	1,198	0 (0%)	–
NMEA	3	18,043	3 (0.02%)	1,198	3 (0.3%)	5
NPYR	2	18,043	41 (0.23%)	1,198	21 (1.8%)	24

Table III. Summary of NDMA Results from UCMR2[11] by Source and Disinfection Type

			Major Disinfection Process		
Sample Type	Water Source	Results	Chloramine (CA)	Chlorine (CL)	Not Identified (NI)
At WTP entry point (EP) to distribution system	Groundwater under the influence	Samples	26	71	4
		Detections	5	0	0
		Percent detected	19	0	0
	Groundwater	Samples	356	4,655	1,316
		Detections	20	60	29
		Percent detected	6	1	2
	Mixed	Samples	85	55	43
		Detections	28	4	7
		Percent detected	33	7	16
	Surface water	Samples	1,139	2,196	653
		Detections	326	87	154
		Percent detected	29	4	24
Distribution system maximum residence time (MR)	Groundwater under the influence	Samples	23	62	1
		Detections	14	1	1
		Percent detected	61	2	0
	Groundwater	Samples	156	1,608	423
		Detections	40	25	7
		Percent detected	26	2	2
	Mixed	Samples	364	639	503
		Detections	161	34	193
		Percent detected	44	5	38
	Surface water	Samples	748	1,790	444
		Detections	402	84	140
		Percent detected	54	5	32

upon measurement of *N*-nitrosamine in various body fluids. Alternatively, estimates can be made based upon levels of products of metabolism of volatile *N*-nitrosamines (e.g., adducts in macromolecules). In the particular case of NDMA, the critical question is whether the *in vitro* model of Krul *et al.*[42] provides a reasonable approximation of endogenous formation compared to those that are derived from other measurements. Estimates based on actual measurements in blood and urine are not constrained, like the model of Krul *et al.*,[42] to reactions that occur within the gastrointestinal tract.

2.3.1. Endogenous Production of Volatile N-Nitrosamines

Generation of endogenous volatile nitrosamines will also depend upon the availability of amine precursors in various body compartments as well as a nitrosating agent. Tricker *et al.*[43] measured the amounts of these precursors in saliva, gastric juice, blood, feces, and urine, finding levels to be similar in gastric juice and blood, and particularly high (except for diethylamine) in urine (Table IV). Based on these measurements, endogenous formation of NDEA would be expected to be more

Table IV. Common Precursors of Volatile *N*-Nitrosamines in Various Body Compartments

Precursor (*N*-Nitrosamine Formed)	Saliva ($n = 20$) mg/L (Range)	Gastric Juice mg/L (Range)	Blood ($n = 10$) mg/L (Range)	Feces ($n = 8$) mg/L (Range)	Urine ($n = 40$) mg/L (Range)
Dimethylamine (NDMA)	0.18 (ND[a]–0.3)	0.87 (0.20–4.2)	0.91 (0.15–2.4)	0.41 (0.1–1.35)	15.9 (2.95–72)
Diethylamine (NDEA)	- (0.03)	0.05 (ND–0.45)	ND	0.03 (ND–0.1)	0.03 (ND–0.7)
Pyrrolidine (NPYR)	0.21 (ND–0.65)	0.18 (ND–0.7)	0.36 (ND–2.2)	0.55 (0.25–1.3)	8.06 (1.0–48.4)
Piperidine (NPIP)	0.49 (ND–1.3)	1.35 (ND–15.6)	1.86 (0.45–2.7)	0.39 (0.2–0.7)	9.35 (1.05–48.4)

Note: Adapted from table I in Tricker *et al.*[43]
[a]ND = not detected.

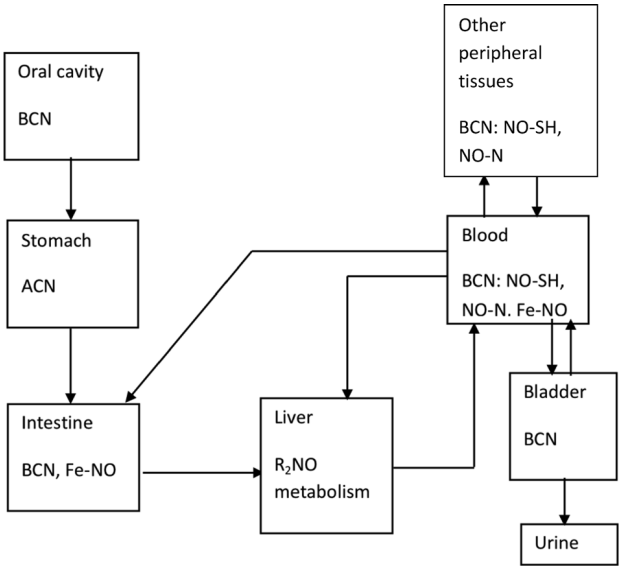

Fig. 1. Potential sources and mechanisms that contribute to endogenous *N*-nitrosamine formation in body compartments. Blood is the central compartment that distributes both ingested and endogenously formed *N*-nitrosamines to all tissues. Urinary concentrations of *N*-nitrosamine reflect the fraction of the nitrosamine that is formed and/or ingested that is cleared by urinary excretion. However, this concentration will be influenced much more by local formation of nitrosamines in the bladder (e.g., associated with bladder infection) than an equivalent amount formed in the stomach. ACN = acid-catalyzed nitrosation, BCN = biologically-catalyzed nitrosation, which can involve multiple intermediates, NO-SH = nitrosothiols, FeNO = iron-nitrosyls, NO-N = *N*-nitrosamines.

dependent upon exogenous precursors than is formation of NDMA. This is consistent with observations in urinary excretion by humans. Factors that influence these concentrations would be likely to influence the types and amounts of *N*-nitrosamines measured in body fluids of any given individual.

An illustration of the different mechanisms of *N*-nitrosamine formation in various body compartments is depicted in Fig. 1. The qualitative interplay in the formation of different volatile *N*-nitrosamines in different compartments should be competitive with other nitrosation reactions. The pK_a of the amine precursor significantly affects the rate of *N*-nitrosation that occurs via the acid-catalyzed mechanism in the stomach.[44] For example, the reaction rates of amino acids such as proline and sarcosine are an order of magnitude greater than those observed for the formation of NDMA, NPIP, and NPYR in the acid conditions of the stomach.[45] The reactions of amino acids will compete for available nitrite. As a result, the amounts of NDMA, NPIP, and NPYR will be reduced if the concentrations of these amino acids are elevated.

The actual pattern of volatile *N*-nitrosamines detected in urine will be influenced by competition among reactions within each compartment. Products formed in the stomach will reflect the variations of the precursor amine and nitrate that are in the diet, but the systemic nitrosation will depend upon the conditions prevailing within each compartment. In the absence of very large increases in dietary intake, the systemic sources are likely to dominate what is observed in the urine. The potential is high for systemic formation as side reactions to the formation of *S*-nitrosothiols, *N*-nitrosamines of amino acids and proteins, and formation of NO-heme derivatives that are observed in diverse tissues.[46] Wu *et al.*[47] associated nitrosation of thiazolidine 4-carboxylic acid with the induction of NOS by acute liver injury. Moreover, transnitro(syl)ation to precursors of volatile nitrosamines can occur systemically as described in Bryan *et al.*[46] Based on increased tumor induction, Lijinsky and Reuber[48] found evidence of a transfer of the nitroso group from proline (noncarcinogenic) to morpholine (carcinogenic) that was catalyzed by thiocyanate. Formation of *N*-nitrosamines in the large intestine is enhanced substantially by heme derived from red meat.[49] Formation of NMOR has been shown to occur in the presence of heme and

nitrite at pH 6.8 *in vitro*, but not in the absence of heme.[50] This reaction would also occur in blood and other tissues with high heme concentrations and tissue levels of NO. Therefore, it seems unlikely that the acid-catalyzed mechanism is the major pathway for endogenous *N*-nitrosamine formation.

There are a variety of pathophysiological conditions associated with inflammation that increase generation of NO and related oxidants.[51] Inflammation that is subclinical, such as obesity-induced inflammation, can also contribute. There are papers in the biomedical literature that have associated various disease states with increased endogenous formation of nitrosamines. This literature is too large to review in detail, but selected papers that associate systemic infections with increased nitrosamine formation in humans and experimental animals are cited to make this point. Leaf *et al.*[52] demonstrated that stimulation of tissues with *Escherichia coli* lipopolysaccharide increased NO synthesis from L-arginine and this was associated with an increase in NMOR in the urine of the rat. Elicited (activated) rat neutrophils nitrosated 2,3-diaminonaphthaline, whereas circulating, nonelicited neutrophils did not.[53] Experiments with woodchucks infected with woodchuck hepatitis virus showed increased NDMA levels in urine by a process that was dependent upon L-arginine.[54] In a follow-up study, a 10-fold increase in the nitrosation of morpholine by infected hepatocytes was found with stimulation by lipopolysaccharide.[55] Ohshima *et al.*[56] found that nitrosamine biosynthesis mediated by nitric oxide synthase was increased by infection with liver flukes. Humans infected with liver flukes were found to have increased nitrosation of proline and thioproline in an extragastric site relative to noninfected controls.[57] Urinary excretion of both volatile and nonvolatile *N*-nitroso compounds was increased in humans infected with schistosomiasis[58] relative to noninfected controls. In Wales, patients who had bladder infections were found to have measurable levels of NDMA, NPIP, or NPYR in their urine, whereas none was detected in 10 control subjects on the same dietary regimen.[59] These data show clearly that systemic formation of *N*-nitrosamines is influenced by disease processes and associated increases in NO production. In a clinical study, increases in eNOS and iNOS occurred in the vascular cells of infants with pulmonary hypertension and congenital heart disease compared with control infants.[60] Therefore, the systemic formation of *N*-nitrosamine in humans is undoubtedly influenced by inflammatory processes and such processes would be expected to increase *N*-nitrosamine formation in infants as well.

This review of the literature shows that the amine substrates are present in body fluids, as well as in the stomach, and that there is a universal availability of nitrosating species (ultimately derived from NO, but likely mediated by transnitrosation of *S*-nitrosothiols and NO-heme derivatives). While there are only limited data for showing actual formation of other volatile *N*-nitrosamines in humans (e.g., NPYR and NPIP in urine), it is highly probable that they are formed systemically as well. Lack of data on their systemic and urinary clearance, however, has precluded our estimation of the actual amounts formed systemically.

2.3.2. Endogenous Production of Volatile N-Nitrosamines in Children

No general description of the relative activity of the NO synthase system during fetal and early postnatal development was located in the literature. However, there are many studies of NO synthase expression and responsiveness of these systems to a variety of specific stimuli in fetal, young, and adult animals.[61–64] While there are insufficient data specific to different age groups to estimate the likely endogenous production in the fetus and young children, there is ample evidence that they are capable of generating NO and, hence, there is a clear potential for synthesizing volatile *N*-nitrosamines. In the absence of age-specific data, we have chosen to scale the formation in these age groups to body weight.

2.3.3. Metabolism of Volatile N-Nitrosamines

Utilization of data on steady-state blood concentrations in humans or daily elimination of volatile *N*-nitrosamines in the urine to estimate endogenous formation is possible, but requires consideration of their rates of clearance from the body. Metabolism is the major mechanism of clearance of the low molecular weight volatile *N*-nitrosamines. Most of the small fraction of NDMA that is not cleared by metabolism is eliminated in the urine.

There are two major pathways of nitrosamine metabolism; hydroxylation (leading to alkylation of nucleophiles) and denitrosation.[65,66] Denitrosation accounts for approximately 20% of NDMA clearance. Hydroxylation is the major pathway that contributes to toxicity and carcinogenicity.[67,68] Both reactions are catalyzed by CYP2E1 (cytochrome p450 2e1; also known as NDMA demethylase).[69] The

mechanisms involved are well described in the literature and will not be reviewed here.

The clearance rate of NDMA has not been studied directly in humans. However, clearance of systemically (as opposed to orally) administered NDMA has been studied in several species. These data indicate that NDMA clearance scales among species as a function of body weight.[70] Using this method of scaling, Gombar *et al.*[70] predicted the clearance of NDMA as 3,450 mL/min for a 70 kg human. Given a blood concentration and evidence that the concentration represents a steady state, this clearance rate is a basis for estimating the rate of endogenous formation of NDMA using conventional pharmacokinetic models.

Aside from sporadic detections, concentrations of other volatile *N*-nitrosamines in human blood were not found in the literature. In addition, their metabolic and urinary clearance has not been established. Therefore, the rates of other volatile *N*-nitrosamines formation could not be estimated.

2.3.4. Metabolism of Volatile N-Nitrosamines in Children

There are substantial age-related differences in the expression of cytochrome P450s and other enzymes that are involved with xenobiotic metabolism. The levels of many of the P450s are not measurable in the fetal liver.[71,72] This is true of CYP2E1, in particular, but its expression increases rapidly after birth. Weanling rats and hamsters actually have higher NDMA demethylase activity than sexually mature rats.[73] A similar pattern would be expected in humans, where the activity of NDMA demethylase reaches adult levels in the first decade of life.[74] Measures of the metabolic clearance of NDMA are needed to estimate age-related rates of endogenous NDMA formation. We were unable to identify such data in the literature.

2.3.5. Excretion of Volatile N-Nitrosamines

Estimating endogenous rates of *N*-nitrosamine formation from their daily excretion in urine is less certain as the amount eliminated in urine is a very small fraction of that which is ingested or formed endogenously. Because volatile *N*-nitrosamines are not significantly eliminated in feces or exhaled air, the remaining clearance is generally attributed to metabolism. The fraction of NDMA that is eliminated in the urine is highly dosedependent.[75] Fig. 2 displays this dose dependence in rats.

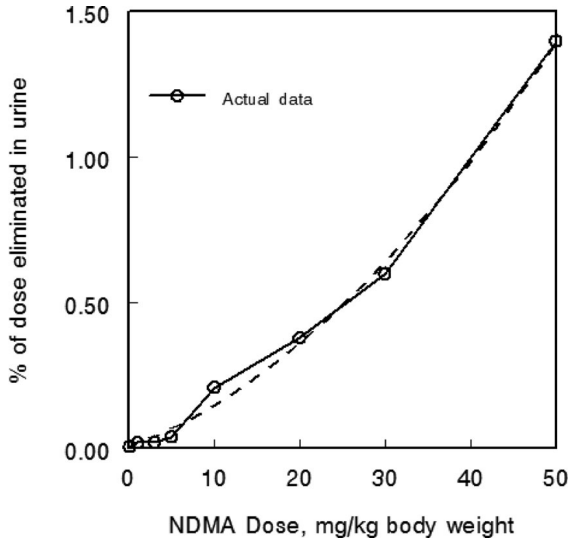

Fig. 2. The recovery of intraperitoneal doses of NDMA in the urine as a function of dose to male Sprague-Dawley rats (data plotted from table 1 of Kraft *et al.*[75]).

At the lowest intraperitoneal dose of NDMA administered (0.1 mg/kg body weight), 0.0064% of the dose was recovered in the urine.[75] Even smaller percentages (0.00049% and 0.00079%) were recovered in the urine of patas monkeys administered a 1 mg/kg body weight intravenous dose of NDMA.[76] In dogs given an intravenous dose of 0.5 mg/kg body weight, NDMA was not detected in the urine (detection limit not provided).[77] It appears that metabolism remains the main clearance mechanism in dogs despite a smaller first pass effect (higher bioavailability) and mean residence time in blood relative to other species. These data are consistent with the observation that less than 0.05% of a dose of NDMA was eliminated in the urine of human volunteers based on the limit of detection for the analysis.[78] Unfortunately, there are insufficient quantitative data on metabolic and urinary clearance of the other volatile *N*-nitrosamines to convert their daily urinary excretion into estimates of their rates of endogenous formation.

2.4. Approaches to Estimating Endogenous Formation of Volatile Nitrosamines

2.4.1. NDMA Concentrations in Human Blood

Several studies are available where blood concentrations of NDMA have been measured in humans,[79–88] and the most useful of these are summarized in Table V. Data from Gough *et al.*[81]

Table V. Mean Concentrations of NDMA in the Blood of Adult Human Volunteers

	N	Mean (ng/kg)	*SD* (ng/kg)	Range (ng/kg)	LoD[a] (ng/kg)
Lakritz *et al.*[83]	38	600	400	0–1,500	100
Simenhoff *et al.*[86]	47	200[b]	270[c]	0–800	50
Gough *et al.*[81]	10	500	160	300–800	150
Lele *et al.*[87][d]	NR[d]	120	137	NR	NR
Simenhoff *et al.*[88]	8	160[e]	1,400[c]	0–500	NR
Dunn *et al.*[84]	58	180	230[c]	0–750	50
Dunn *et al.*[85]	5	20[f]	22[c]	NR	NR

[a]LoD is limit of detection.
[b]Estimated from Fig. 3 in Simenhoff *et al.*[86]
[c]Calculated from standard error of the mean, and sample size as shown in Fig. 8, Simenhoff and Dunn.[89]
[d]NR = not reported.
[e]Estimated from Fig. 5 in Simenhoff *et al.*[86]
[f]Estimated from Fig. 1 in Dunn *et al.*[85]

support the hypothesis that the mean values cited above are approximate steady-state levels as they vary little during the day or for periods as long as three months. While attempts were made to measure other volatile nitrosamines and they were sporadically detected, those data are far too sparse to be useful.

Early measurements of concentrations of NDMA in human blood were plagued by artifacts. These problems were addressed subsequent to the publication of the Lakritz *et al.* study.[83] The remaining papers cited in Table V were published after this was known and these authors strove to rule out artifacts in the analysis. Dunn *et al.*[84] meticulously addressed these questions by specifically analyzing the water used for nitrosamines and suppressing artifactual formation of NDMA in drawn blood by adding ascorbic acid and morpholine (S. R. Dunn, personal communication, 2012). Gough *et al.*[81] explicitly stated that all reagents were checked for contamination, but did not specifically mention the water used. Despite the differences among these studies, the reported values for blood concentrations in the three main studies differ by only a factor of three (excluding Garland *et al.*[90] and Dunn *et al.* ((85)). The second Dunn *et al.*[85] study involved a limited number of subjects (five) in a clinical setting. A study of Garland *et al.*[90] indicated that only nine of 128 plasma samples among 61 individuals had measurable NDMA. Simenhoff and Dunn,[89] as quoted by Lele *et al.*,[87] reported a mean and standard deviation of NDMA concentrations in blood in a control group for people with chronic renal failure, but did not indicate the number of subjects. We concluded that the larger studies of Gough *et al.*,[81] Simenhoff *et al.*,[86] and Dunn *et al.*[84] were more representative samples although results are presented for all estimates in Table V.

2.4.2. Urinary Excretion of NDMA in Humans

Urinary excretion of nitrosamines by untreated human volunteers was reported in a series of experimental field studies. Daily volatile *N*-nitrosamine excretion rates from these subjects are provided in Table VI.

Five of the studies in Table VI were performed in different geographic locations by the same laboratory and using the same analytical methods (the exception is Garland *et al.*[90]). Slight differences in detection limits suggest some refinement of the methods, but the differences among these studies should reflect attributes of the study populations rather than differences in the analyses. Therefore, it seems reasonable that these data indicate significant variation among populations in the nature of endogenous *N*-nitrosamine formation. These geographic differences may be explained by many factors; genetic differences in the processes involved in either nitrosamine formation or metabolism, preexisting disease processes, differences in diet or lifestyle, even the presence of trihalomethanes in drinking water because they are inhibitors/inducers of CYP2E1. The substrates for nitrosation are all endogenous chemicals that appear to be available at significant concentrations (with the exception of diethylamine), but the concentrations can vary widely among individuals (Table IV). Different compartments of *N*-nitrosamine formation would differentially affect the amount that appears in the urine. Because the mechanism of production varies among body compartments, it is also likely that changes in the character of excreted *N*-nitrosamines reflect physiological or pathological conditions within individual compartments (e.g., infected bladder).

Fristachi and Rice[41] focused upon two sources of data for estimating the daily endogenous formation of NDMA. The first was data derived from an *in vitro* gastrointestinal model of NDMA formation.[42] The second method utilized the lowest rate of NDMA elimination by subjects administered alcohol (0.5%)[78] to estimate endogenous formation based on urinary excretion of NDMA in Vermeer *et al.*[93,94] As pointed out by Krul *et al.*,[42] the

Table VI. Nitrosamines Found in the Urine of Nontreated Human Volunteers (ng of NDMA Eliminated/24 Hours)

Study	NDMA	NDEA	NPYR	NPIP	Total
Garland *et al.*[90]a	38.2 ± 24.5[g]	NS[h]	NS	NS	NS
Van Maanen *et al.*[91]b	11.3 ± 43.4	13.4 ± 40.4	90.5 ± 77.6	38.3 ± 78.4	153 ± 136
"detections only (*n*)"	124 ± 107 (2)	98 ± 68 (3)	105 ± 70 (18)	140 ± 73 (6)	178 ± 131
Van Maanen *et al.*[92]c	89 ± 22	ND[i]	ND	ND	
Vermeer *et al.*[93]d	287 ± 223	ND	ND	69 ± 36	
Vermeer *et al.*[94]e	385 ± 196	ND	ND	117 ± 75	
Levallois *et al.*[95]f	ND	ND	ND	67 ± 90	

[a]$n = 24$. [b]$n = 24$. [c]$n = 48$. [d]$n = 25$. [e]$n = 25$. [f]$n = 59$. [g]Mean ± *SD*. [h]NS = not sought. [i]ND = not detected.

amount of NDMA predicted from the *in vitro* model system is substantially lower than the estimates that are derived from the studies of urinary elimination of NDMA and is not an appropriate basis for estimating NDMA formation in the entire body. Second, alcohol is a potent inhibitor of NDMA metabolism. Anderson *et al.*[76] found that the urinary elimination of NDMA increased 100–800-fold when a single dose of NDMA was administered simultaneously with ethanol or isopropanol. Speigelhalder *et al.*[78] found that NDMA was not measurable in the absence of ethanol treatment of humans and indicated, based upon their detection limits, that urinary clearance had to be below 0.05% of the dose in individuals not administered alcohol.

2.4.3. DNA Adducts Associated with NDMA Exposure

The existence of adducts in human DNA in the absence of obvious exposure to exogenous electrophiles became apparent in the 1990s.[96] Prominent among these are methylated adducts produced by NDMA.[97] Much of the O^6-methylguanine (O^6-MeG) adduct found in human DNA has been postulated to arise from endogenous production of NDMA.[98] Therefore, if the doseresponse for producing these adducts, their steady-state levels, and their rate of repair in a specific tissue can be established, the amount of endogenous NDMA required to generate those levels could be estimated.

The levels of O^6-MeG vary widely in humans by tissue.[97] Although fewer adducts are observed in leukocytes of humans, these are much more readily sampled than human liver. Georgiadis *et al.*[99] utilized a methylated oligonucleotide as an internal standard in samples of DNA drawn from 36 maternal and cord blood samples. The method was based upon competitive repair of the adducts in the synthetic oligonucleotide and in the sample by O^6-alkylguanine-DNA alkyltransferase (the enzyme is inactivated as it removes the alkyl group from the nucleotide). Thirty-one of 36 maternal blood samples had a mean of 56 with a range of 16–176 nmol O^6-MeG/mol guanine (G). The corresponding value in 30 of 36 cord blood samples was 45 with a range of 16–192 nmol O^6-MeG/mol G. While mean levels in the cord blood were lower than the maternal levels, the results were significantly correlated with one another ($r^2 = 0.47$, $p < 0.0001$). These adduct levels could not be associated with any known source of external nitrosamine exposure.

Georgiadis *et al.*[100] published a second study of O^6-MeG adducts in 120 pairs of maternal and cord blood DNA samples utilizing a new immunochemical assay that analyzed digested DNA using an antiss-DNA antisera. One hundred and twenty maternal and cord blood pairs were analyzed, of which 70% of the maternal and 50% of the cord bloods were found to have measurable amounts of O^6-MeG adducts. Means of 0.65 and 0.38 adducts/10^8 nucleotides were found in maternal and cord blood samples, respectively. Approximately 20% of the bases in human DNA are guanine.[101] Therefore, the results of the second Georgiadis study[100] can be normalized with the first by simply multiplying by 5 and then by 10 (to convert to a denominator of 10^9) to arrive at 32.5 O^6-MeG/10^9 G. Therefore, the mean values of the second study are about 63% of those observed in the first study. This may be due, in part, from exclusion of smokers from the second study.

The half-life of O^6MeG in human white blood cells is approximately 22–25 hours.[99] O^6-MeG adducts in the maternal blood of pregnant patas monkeys given an oral dose of 0.1 mg NDMA per kg body weight were 240 nmol O^6-MeG/mol G.[102] The kinetics of O^6-MeG accumulation in hepatic and leukocyte DNA of rats have been studied with

extended treatments with low doses of NDMA (0.2–2.64 mg/L of drinking water) for 2–180 days.[103,104] These treatments did not affect the level of the O^6-alkylguanine-DNA alkyltransferase, the repair enzyme that removes the O^6-MeG adduct from DNA, indicating that doses in this range do not overwhelm the repair capacity of these tissues. Repair occurred with a half-life of 20–25 hours. Consistent with this half-life, the adduct level reached a steady state in rats within eight days.[104]

NDMA is not the only compound that methylates DNA. Therefore, using levels of O^6-MeG as a basis for calculating endogenous dose of NDMA is valid only in the substantial absence of exposures to these other agents. A list of such agents is provided in Table VII. Exposure to such chemicals is likely to occur from a limited variety of sources. Some are used in industry for methylation reactions in synthetic chemistry and others are commonly used in mechanistic studies in research laboratories. These are the more likely occupational exposures. At least one of these compounds can also be formed endogenously (*N*-methy-*N*-nitrosourea). A variety of chemotherapeutic agents methylate DNA and another methylating *N*-nitrosamine is associated with the use of tobacco. The relative efficiency of these agents in specifically producing O^6-meG adducts relative to methylation of other sites within DNA differs substantially depending upon their reactivity.

3. METHODS

3.1. Modeling Cumulative Exposure to NDMA Among Nonsmokers

In nonsmokers who are not occupationally exposed, the main sources of NDMA are endogenous synthesis, food, and drinking water. Though dermal uptake from water or from cosmetics is possible, the potential amounts involved are likely to be negligible.[105]

Contributions from each source were modeled separately (see Fig. 3). Results in terms of average daily dose (ADD) were obtained for each of eight age cohorts, and a lifetime average daily dose (LADD) was calculated assuming a 75-year lifespan. The proportion of intake (POI, i.e., exogenous only), and of exposure (POE, i.e., all sources including endogenous formation) due to drinking water was estimated for both the ADD and the LADD.

3.1.1. Modeling NDMA Exposure from Endogenous Synthesis

We have pursued the following approaches for estimating endogenous exposure of NDMA: (1) based on reported steady-state blood levels of NDMA, (2) based on reported levels of O^6–meG adducts in DNA that result from metabolism of NDMA, and (3) based on reported urinary excretion in conjunction with literature values for urinary NDMA levels.

3.1.1.1. Estimating NDMA exposure based on blood levels. Gombar *et al.*[70] considered that the measured level of NDMA in the blood of a fasted animal represented a steady-state concentration, and estimated the rate of clearance of the substance in humans at 3.45 L/min, based on the finding that clearance scaled by body weight across species.[57] From these data, it is possible to estimate a rate of synthesis of NDMA necessary to maintain the measured concentrations in the blood. This is assumed to be largely due to endogenous synthesis, as the estimated rate is much larger than possible from exogenous intakes.

Of the literature values for NDMA blood levels (Table V), the earliest[70] was discarded because of uncertainty about whether the potential for artifacts[79] had been considered. The remaining authors reported (mean) levels ranging from 0.02 to 0.5 μg/L. The model uses the values for steady-state blood NDMA levels from these sources to provide a range of estimated endogenous formation, and hence a range of potential ADDs and LADDs.

From the formula $k_0 = C_{ss} \times Cl$, the infusion rate (k_0, μg/min) of NDMA into the systemic blood can be estimated from the value of steady-state concentration (C_{ss}, μg/L) and the clearance rate (Cl) of 3.45 L/min. Our model applies the dose (in μg/kg/day) thus obtained to all age cohorts. This assumption that the per kg rate of synthesis is constant through life was necessary in part due to the lack of data on blood levels of NDMA at different ages.

3.1.1.2. Estimating NDMA exposure based on levels of DNA adducts in leukocytes. Souliotis *et al.*[104] reported that steady-state levels of O^6-meG DNA adducts were linearly related to dose in the rat, with no adducts present in the zero-dose condition. Georgiadis *et al.*[100] reported levels of O^6-meG in maternal and cord blood (i.e., leukocytes) of a

Table VII. Examples of Methylating Agents

General Use	Methylating Agents	Exposure
Reagent chemicals	Dimethyl sulfate	Occupational
	Methyl iodide	Occupational
	Dimethyl carbonate	Occupational
	Methyl triflate	Occupational
	Methyl flourosulfonate	Occupational
Biochemical tools	*N*-Methyl-*N*′-nitro-*N*-nitrosoguanidine	Occupational
	N-Methyl-*N*-nitrosourea	Endogenous
	N-Nitroso-*N*-dimethylamine	Endogenous, diet
	Methyl methane sulfonate	Occupational
Cancer chemotherapy	Procarbazine	Therapy
	Dacarbazine	Therapy
	5-(3-methyl-1-triazeno)imidazole-4-carboxamide	Therapy
	Temozolomide	Therapy
	Streptozotocin	Therapy
Tobacco	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone	Smoking/chewing

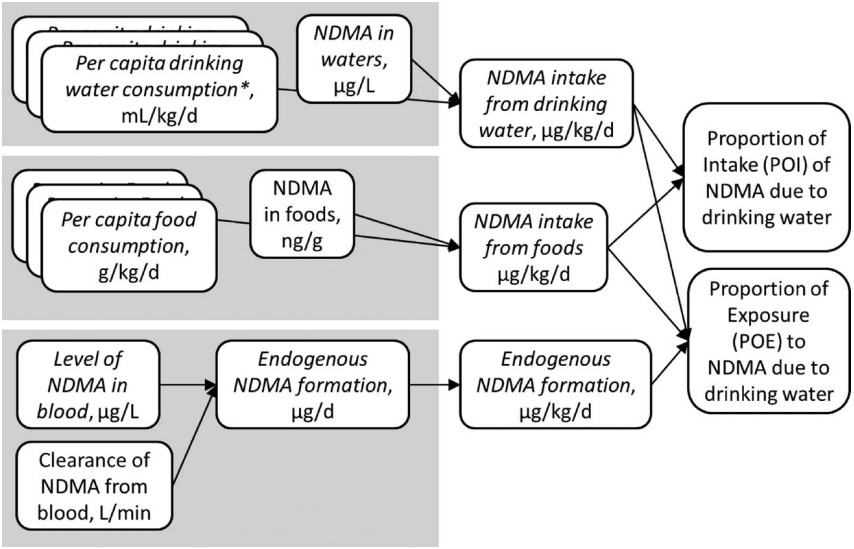

Fig. 3. Structure of our model. Italics indicate distributions whereas regular type indicates fixed values. Overlapping elements represent values specific for each of eight age cohorts. NDMA intake from food was obtained by summing over 10 food categories.

population of Greek mothers and their newborns. These mothers were believed to have low exposure to exogenous sources of NDMA. The mean and maximum levels of O^6-meG in human leukocytes[100] were used to find the corresponding steady-state oral dose in rats that produces the same level of the adduct[104] as described in Section 2.4.3.

3.1.1.3. Estimating NDMA exposure based on urinary excretion. There is considerable qualitative and quantitative variation in reports of volatile *N*-nitrosamines excreted in urine by humans (see Table VI). Our estimate of exposure is based on the assumption that the fraction of NDMA excreted in humans is similar to that reported in rats. As indicated previously, this figure was intermediate between the maximum urinary clearance in humans in the absence of ethanol treatment and a much lower rate of clearance observed in a primate model.

The amount of NDMA excreted in 24 hours was divided by the excretion fraction in rats dosed intraperitoneally at 0.1 μg/kg (0.00006).[75] This rate of elimination was applied to the human urinary excretion data to estimate daily endogenous formation of NDMA.

3.1.2. Modeling NDMA Exposure from Diet

3.1.2.1. Concentration of NDMA in foods. Several authors have compiled data for levels of NDMA in

Table VIII. Inputs to the Dietary Exposure Module with Consumption Data Presented for Two of the Eight Age Cohorts

		Total Diet Study[111]		Exposure Factors Handbook[112]	
Food Category	Biaudet *et al.*[109] NDMA (ng/g)	6–12 Years (g/day)	20–49 Years (g/day)	6–12 Years (g/kg/day)[a]	20–49 Years (g/kg/day)[b]
Whole milk	0.03	111.7	49.8	4.7	1.1
Skim/low fat milk	0.03	282.1	110.7	7.6	2.2
Cheese	0.32	16.5	20.3	0.5	0.2
Beef	0.10	52.4	77.2	1.1	0.7
Pork	0.28	16.8	21.5	0.5	0.4
Poultry	0.00	31.4	51.0	1.2	0.7
Fish and seafood	0.37	8.7	17.2	0.2	0.2
Cereal[c]	0.11	236	251	6.3	3.2
Beer	0.20[d]	0.0	129.6	0.0	1.7
Breast milk	0.14[e]	0.0	0.0	0.0	0.0
Formula	0.0544[f]	0.0	0.0	0.0	0.0

Note: Concentrations shown are mean values.
[a]Mean body weights are 31.8 kg for 6–12-year olds.
[b]Mean body weights are 80 kg for 20–49-year olds.
[c]Cereals include bread, pasta, rice, cakes and pastries, and dry cereal.
[d]NDMA concentrations in beer were taken from Baxter *et al.*[110]
[e]NDMA concentrations in breast milk were based on Lakritz and Pensabene[82] and on Uibu *et al.*[113]
[f]NDMA concentrations in formula were based on Jurado-Sànchez *et al.*[114]

foods, with original sources dating back to 1980 and earlier.[106–108] After early reports of nitrosamines in foods and elucidation of the mechanism of formation, measures to reduce this exposure caused concentrations of NDMA in foods to drop approximately 10-fold over a decade.[105] The model, therefore, incorporates data from Biaudet *et al.*[109] that is based on measurements made over the period 1987–1992, to represent the concentration of NDMA in various foods. Levels in formula and breast milk were not provided by this reference and were sought elsewhere (as described in Section 3.1.2.4); levels in beer were obtained from a recent survey by Baxter *et al.*[110] Consumption of different NDMA sources in the diet was approached on a *per capita* basis using data from the Total Diet Study Version 3 (TDS-V3),[111] and from the Exposure Factor Handbook 2011[112] (EFH) (Table VIII).

3.1.2.2. Per capita dietary intake from the Total Diet Study. *Per capita* consumption amounts were obtained from the TDS-V3, which is intended to provide an exhaustive description of a typical diet in the United States, using data from the 1994–1996, 1998 CSFII.[111] Amounts are provided by the TDS-V3 in g/day for consumption of specific dishes by different age cohorts with the exception of birth to six months. For modeling purposes, total amounts of each food category were obtained by summing the amounts reported for the food itself (i.e., as a main dish), plus arbitrary fractions of "mixed foods" of which the food forms a component. For example, cheese was considered to comprise 0.1 of a serving of macaroni and cheese, and milk was assumed to comprise 0.25 of the same serving. The estimate of NDMA intake in this approach is therefore the product of the *per capita* amount consumed (fixed value), and the concentration of NDMA in the food (fixed value). The youngest age class in the TDS is 6–11 months, so this approach did not include the birth to six months cohort.

3.1.2.3. Per capita dietary intake from the Exposure Factor Handbook. The Exposure Factor Handbook 2011[112] provided *per capita* consumption data in g/kg/day, based on data from the 2003–2006 NHANES, and derived by averaging "consumers-only" amounts across the entire population. Estimated NDMA intake is the product of the amount of each food consumed in a day (lognormal distribution), and the concentration of NDMA in that food (fixed value), summed over all foods. The POI and POE results presented herein utilize the dietary intake estimate based on these EFH 2011 *per capita* data.

3.1.2.4. Dietary intake of infants. The model provides a choice of three conditions to represent infants in the age range of birth to six months: (1) exclusively formula-fed, (2) exclusively breast-fed, or (3) nonexclusive, representing a population whose diet ranges between exclusively formula-fed and exclusively breast-fed. The probability of consuming breast milk or formula is equivalent for infants at the midpoint of the cohort, three months of age.[115] In addition, a small amount of cereal is consumed by some infants in this cohort.

The level of NDMA in breast milk is assigned the average of estimates from Lakritz and Pensabene[82] and Uibu *et al.*[113] No data were found reporting concentration of NDMA in infant formula specifically. Most formulas given to infants who are not breastfeeding are based on cow's milk.[116] The U.S. Food and Drug Administration does not specifically regulate the amount of nitrosamines in milk. A range of estimates was used to represent potential levels of NDMA in formula, with a low value calculated from NDMA levels reported in milk proteins[117] and the proportion of milk proteins in reconstituted formula, and three higher values based on reports of NDMA in samples of nonfat dry milk[114,118,119] and the proportion of this ingredient in prepared formula. The levels thus calculated (in prepared formula) were 0.0015, 0.02, 0.05, and 0.06 ng/g, respectively. The results presented herein are based on the concentration estimate (0.02 ng/g) from Jurado-Sànchez *et al.*,[114] which is the most recent available.

3.1.3. Modeling NDMA Exposure via Drinking Water

Based on maximum likelihood estimation, a series of lognormal distributions were defined to represent the concentration of NDMA in the range of drinking water source types characterized by sampling point, source water, and disinfection method.

Consumption distributions were constructed using recommended values from EFH 2011,[112] for combined direct and indirect water from community water supply, *per capita*, based on a U.S. EPA analysis of NHANES 2003–2006 data. Like the dietary values, these were derived by averaging "consumers-only" amounts over the entire population, and were in units of mL/kg/day. Lognormal distributions were assumed. For each age cohort and each water source type, the estimated NDMA intake is the product of the distribution of drinking water consumption for the age, and the distribution of NDMA concentration in that water source type within a Monte Carlo simulation.

Table IX. Estimates of Endogenous Exposure to NDMA in Terms of Amount per Day and as μg/kg Body Weight in Adults

	Endogenous NDMA (μg/day)			Endogenous NDMA (μg/kg/day)		
	Median	Mean	95th%	Median	Mean	95th%
Simenhoff *et al.*[86]	590	990	3,160	8.4	14	45
Gough *et al.*[81]	2,310	2,480	4,350	33	35	62
Gough *et al.* corrected[a]	1,240	1,490	3,360	18	21	48
Lele *et al.*[87]	390	600	1,780	5.5	8.5	25
Simenhoff *et al.*[88]	90	790	2,790	1.3	11	40
Dunn *et al.* 1986[84]	550	890	2,780	7.9	13	40
Dunn *et al.* 1990[85]	70	100	290	0.9	1.4	4.1

[a]The mean and *SD* were corrected by subtracting the level reported for the water blank.

4. RESULTS

4.1. Endogenous Input

Applying a clearance rate of 3.45 L/min, we obtained a range of estimated mean NDMA infusion rates from 100 to nearly 2,500 μg/day (Table IX). The corresponding dose range expressed per unit body weight is from 1.4 to 35 μg/kg body weight per day, assuming a weight of 71.5 kg for the adult subjects providing these data (from the EFH 1997[120]). These values represent systemic exposure of adults to NDMA, and include NDMA formed in the gastrointestinal system, or preformed NDMA ingested or otherwise absorbed, but the latter amounts are negligible by comparison.

The estimated endogenous NDMA dose based on levels of O^6-meG in leukocytes corresponded to 18 μg/kg/day (*ca.,* 1,360 μg/day) for the mean and 220 μg/kg/day (*ca.*, 17,000 μg/day) for the maximum. These values estimate endogenous exposure in the human subjects under the assumption that the kinetics of NDMA in the rat is substantially similar to those in humans. This estimate does not incorporate a calculation for first pass metabolism in the liver because the leukocytes in the blood are exposed to the NDMA in the portal circulation before it reaches the

liver. As noted previously, NDMA is not the only chemical exposure that can lead to formation of O^6-meG adducts in DNA. Therefore, it is not surprising that this estimate of endogenous NDMA dose falls in the upper range of estimates made from blood concentrations.

Finally, the estimated formation of endogenous NDMA based on urinary excretion ranged from <250 to 6,400 μg/day (<4 to 90 μg/kg/day) depending on the population from which the excretion data were obtained. Estimates of formation of other volatile *N*-nitrosamines were not possible because quantitative data on which to estimate metabolic and urinary clearance rates were not available.

4.2. Exogenous Inputs

Although we considered available evidence on several volatile *N*-nitrosamines known to occur in drinking water, the data indicate that only NDMA is detected in a substantial proportion of water systems and data on other volatile *N*-nitrosamines in diet or produced by endogenous production are inadequate to develop a comparative exposure model for these *N*-nitrosamines.

4.2.1. Dietary Exposure

Based on *per capita* consumption data, the daily mean exposure to dietary NDMA is estimated to range from 0.7 to 11 ng/kg/day, depending on age, with adults aged 20–49 years experiencing a mean exposure of 0.8 ng/kg/day (approximately 60 ng/day). If beer is included in the adult diet the intake is 1 ng/kg/day (results not shown). The 95th percentile of intake is 1.4 ng/kg/day in adults aged 20–49 years (or 2.1 ng/kg/day if beer is included), whereas during the first six months of life (fed a nonexclusive diet) the 95th percentile of intake is estimated at 14.6 ng/kg/day (Table X).

In exclusively formula-fed infants aged birth to six months, the dietary exposure to NDMA arises from the level of the chemical in the dry powder since the water used to reconstitute the formula is included under drinking water. The mean estimated dietary intake of NDMA in exclusively formula-fed infants is 6.9 ng/kg/day, as compared to an estimate of 15 ng/kg/day in exclusively breast-fed infants (results not shown).

4.2.2. Drinking Water Exposure

Samples of drinking water sourced from groundwater exhibited a lower rate of detections of NDMA, and a lower mean concentration of NDMA than did samples from surface water, as expected. Similarly, regardless of water source, samples from systems using chloramination had a higher rate of detection and higher mean levels of NDMA than samples from systems using free chlorine (Fig. 4).

Table X. Statistics of NDMA Intake from Diet in ng/kg/day, Based on the Total Diet Study[111] or the Exposure Factors Handbook[112] (Both Based on *Per Capita* Rates)

Age (years)	Total Diet Study (ng/kg/day) Median	Exposure Factors Handbook (ng/kg/day) Median	Mean	95th%
0 to <0.5	(no data provided)	11	11	14.6
0.5 to <1	2.1	6.5	6.5	8.9
1–2	3.7	2.9	3.0	5.0
3–5	2.7	2.2	2.4	4.0
6–12	1.8	1.4	1.6	2.8
13–19	1.1	0.8	0.9	2.0
20–49	0.7	0.7	0.8	1.6
50 and up	0.7	0.6	0.7	1.4

Note: The infant (0 to <0.5 years) diet condition represented here is "nonexclusive."

Based on distributions of NDMA concentration in drinking water from the UCMR2 data combined with *per capita* drinking water consumption from the EFH 2011, we estimate that the mean daily NDMA intake associated with surface water treated with chloramine is approximately eightfold higher than the intake from surface water treated with chlorine (Table XI). Given similar disinfection treatment, groundwater is associated with a lower daily NDMA intake than is surface water, as expected. The absolute intake of NDMA from drinking water in infants aged birth to six months is comparable to the intake in children 6–12 years, and for exclusively formula-fed infants the NDMA intake from drinking water is higher than that in children 6–12. (Infants who are exclusively breast-fed are assumed to have no direct contribution from drinking water over the period from birth to six months.)

Table XII provides sampling data for the source types, and NDMA intake rates for three cohorts. The youngest cohort experiences different rates of NDMA intake depending on the source of nutrition because tap water is often used to prepare formula.

4.3. Other Exposure Sources Not Included in Model

We have not included inhalation sources for NDMA because there is limited information of any significant inhalation sources affecting a substantial

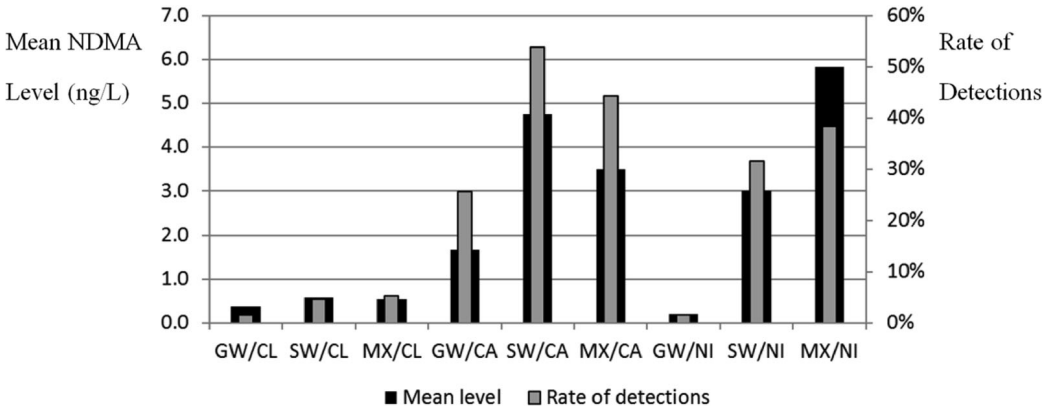

Fig. 4. Mean NDMA concentration and prevalence in drinking water by source type. Water types are groundwater (GW), surface water (SW), and mixed (MX). Disinfection methods are free chlorine (CL) and chloramine (CA); NI represents samples for which a disinfection method was not indicated. All samples were taken at maximum residence time (distribution system). For the NI sources, MX were higher than SW, a counterintuitive result because of a wider distribution of results in MX systems.

Table XI. NDMA Intake from Drinking Water in Three Age Cohorts for Two Water Source Types

	Mean NDMA Intake (ng/kg/day)			95th Percentile NDMA Intake (ng/kg/day)		
	0–0.5 Years	6–12 Years	20–49 Years	0–0.5 Years	6–12 Years	20–49 Years
SW/CL/MR	0.05 [0.07]	0.008	0.007	0.12 [0.21]	0.02	0.02
SW/CA/MR	0.37 [0.57]	0.07	0.06	1.6 [2.1]	0.26	0.23

Note: Values in the 0–0.5 year cohort are for the nonexclusive diet condition, with values for exclusively formula-fed infants following in brackets.

proportion of the population other than tobacco smoke. To the extent that any other sources of NDMA exposures, including smoking or secondhand smoke, do occur, the proportion of total exposure to NDMA attributable to drinking water will be smaller than the estimates presented by our model.

4.4. Cumulative Exposure to NDMA

Tables XIII–XV present estimates for the contribution of drinking water to the average daily exposure or the lifetime average daily exposure in terms of exogenous sources only (POI) or in terms of all sources (POE). Results are presented for endogenous estimates based on Dunn *et al.*,[84] and on Dunn *et al.*,[85] which span the range of the most recent reported human blood NDMA levels.

The POE LADD calculation is a simple ratio of drinking water intake over total NDMA exposure. Therefore, increases in either consumption of drinking water or NDMA concentration in water result in proportional increases in the POE LADD, as discussed in Appendix A. Similarly, the POE LADD estimate can be decreased by increasing the size of the denominator (total NDMA exposure), of which endogenous formation comprises the largest part.

5. DISCUSSION

5.1. Endogenous Formation

Our analyses clearly show that the largest exposure to NDMA (and, by extension, to other volatile *N*-nitrosamines) arises from endogenous processes. This has been recognized in the biomedical literature for some time. We utilized three types of data to estimate the extent of daily endogenous NDMA formation in humans. Considering the divergent nature of the data, the estimates are in remarkable agreement (Section 4.1; Table XI). The mean estimated endogenous NDMA formation based on analysis of blood of human volunteers for NDMA was approximately 900 μg/day when estimated using the blood NDMA level from Dunn *et al.*[84] The estimated mean endogenous

Table XII. Characteristics of the Water Sources and Daily NDMA Intake for Three Cohorts

Water Source Characteristics				Mean NDMA Intake from Drinking Water, for Distribution System Exposure Scenario (ng/day)			
	Samples	Detects	Mean NDMA Level (ng/L)	Birth to Six Months, (Nonexclusive Diet)	Birth to Six Months, (Formula-Fed Only)	6–12 Years	20–49 Years
GW/CL/EP	4655	60	0.17				
GW/CL/MR	1608	25	0.37	0.2	0.3	0.2	0.4
SW/CL/EP	2196	87	0.46				
SW/CL/MR	1,790	84	0.57	0.2	0.4	0.3	0.6
MX/CL/EP[a]	55	4	1.27	0.6	0.9	0.6	1.4
MX/CL/MR	639	34	0.54	0.3	0.4	0.2	0.5
GW/CA/EP	356	20	0.61				
GW/CA/MR	156	40	1.67	0.8	1.2	0.7	1.7
SW/CA/EP	1,139	326	2.87				
SW/CA/MR	748	402	4.76	2.2	3.3	2.1	5.1
MX/CA/EP[a]	85	28	2.63				
MX/CA/MR	364	161	3.50	1.6	2.5	1.6	3.7
GW/NI[b] /EP	1,316	29	0.43	0.2	0.3	0.2	0.5
GW/NI[b]/MR	423	7	0.21	0.1	0.2	0.1	0.2
SW/NI[b]/EP	365	154	4.92	2.3	3.5	2.2	5.1
SW/NI[b]/MR	444	140	3.01	1.4	2.1	1.3	3.1
MX/NI[b]/EP [b]	43	7	1.29				
MX/NI[b]/MR	503	193	5.83	2.6	4.1	2.6	6.1

Note: GW = groundwater; SW = surface water; MX = mixed; CL = free chlorine; CA = chloramine; blank = disinfection method not stated; EP = entry point; MR = maximum residence time. NDMA intakes are shown only for MR (distribution system) sites unless EP concentrations were higher.

[a]Fewer than 100 samples in category.

[b]NI means disinfection process was not identified for these facilities.

Table XIII. Comparison of Mean POI (Exogenous NDMA Only) and Mean POE (All NDMA Exposure Sources) in Terms of the Average Daily Dose (ADD) of NDMA, for Exclusively Formula-Fed Infants Aged Birth to Six Months, Based on Either the Mean or the 95th Percentile of Drinking Water NDMA Intake

	POI (Food, Water NDMA Ingestion) from Drinking Water with Respect to the ADD		POE (Compared to All NDMA Sources) from Drinking Water with Respect to the ADD	
Formula-Fed Infants Infants (Birth to Six Months)	Based on Mean Intake	Based on 95th Percentile Intake	Based on Mean Intake	Based on 95th Percentile Intake
GW/CL/MR	0.6%	2.2%	0.001% / 0.01%	0.003% / 0.02%
GW/CA/MR	2.7%	8.6%	0.004% / 0.03%	0.01% / 0.10%
SW/CL/MR	0.7%	2.9%	0.001% / 0.01%	0.004% / 0.03%
SW/CA/MR	6.2%	24%	0.01% / 0.09%	0.04% / 0.3%
MX/CL/MR	0.8%	3.5%	0.001% / 0.01%	0.005% / 0.04%
MX/CA/MR	4.8%	19%	0.008% / 0.06%	0.03% / 0.2%

Note: Results are based on 0.0544 ng/g NDMA in formula, and POE are given for upper and lower blood NDMA levels either as per Dunn *et al.*,[84] or Dunn *et al.*[85]

NDMA formation based on the lower blood NDMA level from Dunn *et al.*[85] was 100 μg/day with an upper 95th percentile value of 290 μg/day. This lower end of the range of estimates for endogenous NDMA formation was based on a very small sample size ($N = 5$). A mean estimate based upon levels of O^6-MeG in DNA derived from human blood was 1,360 μg/day, ranging up to a high of 17,000 μg/day. The estimated amount of endogenous NDMA formation based on urinary excretion ranged from <250 to 6,400 μg/day using the fraction of a systemic dose that is cleared by the kidneys in rats.

Table XIV. Comparison of Mean POI (Exogenous NDMA Only) and Mean POE (All NDMA Exposure Sources) in Terms of the Average Daily Dose (ADD) of NDMA, for Adults 20–49 Years, Based on Either the Mean or the 95th Percentile of Drinking Water NDMA Intake

	POI (Food, Water NDMA Ingestion) from Drinking Water with Respect to the ADD		POE (Compared to All NDMA Sources) from Drinking Water with Respect to the ADD	
Adults (20–49)	Based on Mean Intake	Based on 95th Percentile Intake	Based on Mean Intake	Based on 95th Percentile Intake
GW/CL/MR	0.8%	2.9%	0.0001% / 0.001%	0.0004% / 0.003%
GW/CA/MR	3.2%	11%	0.0005% / 0.003%	0.002% / 0.01%
SW/CL/MR	0.9%	3.5%	0.0001% / 0.001%	0.0004%/ 0.003%
SW/CA/MR	7.0%	27%	0.001% /0.01%	0.005% / 0.04%
MX/CL/MR	1.0%	4.2%	0.0002% / 0.001%	0.0006% / 0.004%
MX/CA/MR	5.5%	22%	0.0009% / 0.007%	0.004% / 0.03%

Note: Results are based on POE given for upper and lower blood NDMA levels either as per Dunn *et al.*[84] or Dunn *et al.*[85]

Table XV. Comparison of Mean POI (Exogenous NDMA Only) and Mean POE (All NDMA Exposure Sources) in Terms of the Lifetime Average Daily Dose (LADD) of NDMA in Those Exclusively Formula-Fed as Infants, Based on Either the Mean or the 95th Percentile of Drinking Water NDMA Intake

	POI (Food, Water NDMA Ingestion) Due to Drinking Water with Respect to the LADD		POE (Compared to all NDMA Sources) Due to Drinking Water with Respect to the LADD	
(Lifetime)	Based on Mean Intake	Based on 95th Percentile Intake	Based on Mean Intake	Based on 95th Percentile intake
GW/CL/MR	0.54%	2.0%	0.0001% / 0.001%	0.0004% / 0.003%
GW/CA/MR	2.3%	7.7%	0.0005% / 0.004%	0.002% / 0.01%
SW/CL/MR	0.65%	2.4%	0.0002% / 0.001%	0.0005% / 0.004%
SW/CA/MR	5.5%	21%	0.001% / 0.01%	0.005% / 0.04%
MX/CL/MR	0.73%	3.0%	0.0002% / 0.001%	0.0006% / 0.005%
MX/CA/MR	4.3%	16%	0.001% / 0.008%	0.004% / 0.03%

Note: Results are based on 0.0544 ng/g NDMA in formula, and POE are given for upper and lower blood NDMA levels either as per Dunn *et al.*[84] or Dunn *et al.*[85]

The accuracy of these results depends not only on the accuracy of the measurements, but on pharmacokinetic and metabolic data that were not altogether specific to humans. The estimate of overall clearance of NDMA was based on extensive data in several animal species and as a consequence is sufficiently reliable for purposes of estimating likely synthesis rates in adult humans. Moreover, metabolism of volatile *N*-nitrosamines is modified by many factors that have not been explicitly quantified in humans. The interspecies scaling factor used was simply proportional to body weight.[70] Although we were more or less forced to assume the intraspecies scaling followed the same rule, the limited data that are available suggest this might not be the case. Thus, it should not be assumed that the scaling by body weight can be simply used to estimate within- species variation, especially relative to developmental age. There are data to suggest variations of metabolic enzymes important to the clearance of NDMA may not scale to the very young. On the other hand, our overall conclusions may not be sensitive to variations in metabolism by the young. Virtually all of the clearance of NDMA is still via metabolism because very small fractions of NDMA doses appear in the urine of all species despite the wide variation in first-pass metabolism. This suggests that as a very small molecule, NDMA is not efficiently concentrated in

the urine for elimination (i.e., is largely reabsorbed). Therefore, elimination of NDMA will still remain dependent upon the metabolism that activates it as a carcinogen. It just takes longer if the key enzyme is at a lower level.

There is also a question of how representative these data might be of the human population. The basis of variation of the predominant volatile *N*-nitrosamines eliminated in the urine among populations was not apparent in the blood or DNA adduct measurements. Only NDMA or its DNA adduct has been measured in subjects considered to have minimal exogenous exposure. Nevertheless, these data are consistent with the variation in the blood or O^6-MeG levels, which also range from a significant number of nondetects to relatively high concentrations. This suggests a wide variation in the formation of each volatile *N*-nitrosamine formed among individuals. However, it must be pointed out that many factors affect *N*-nitrosamines in urine, including genetic, lifestyle, and environmental variables that will modify rates of metabolism. None of the studies addressed the factors that could have substantial effects on the rate of metabolism, and the large multipliers used to calculate the dose required to produce the measured amount in urine also multiply errors. However, using the most conservative multiplier based on 0.05% of a systemic dose being eliminated in the urine would still result in estimates that exceed the doses obtained from food or drinking water by orders of magnitude.

The estimate of endogenous formation by measurement of methylated DNA bases in blood is also subject to the same errors that would be observed with measurements of the parent compound. However, the calculation also assumes that the only methylating agent to which these individuals were exposed was NDMA. NDMA is very efficient at methylating DNA and exposure to it from endogenous formation is well established, but it is not the only agent that forms such adducts. Needless to say, if any of the subjects were exposed to such agents, this would inaccurately contribute to the projected formation rate.

Despite the multiple possible sources of error in the estimates of endogenous formation of NDMA we have derived, it seems clear that formation rates approaching 1 mg/day are usual and in some individuals such formation can be significantly greater. Notably, Tannenbaum[121] estimated that up to 670 μg/day of NDMA was formed endogenously using similar methodology.

5.2. Dietary Intake

We estimate that the daily dietary intake of NDMA in the U.S. population ranges from 0.03 to 0.06 μg/day, depending on age, with adults aged 20–49 years experiencing an exposure of 0.06 μg/day (or 0.08 μg/day when beer is included). In comparison, others have reported dietary intakes of 0.17 μg/day (in women) and 0.28 μg/day (in men) in West Germany,[122] 0.19 μg/day in France,[109] 0.05 μg/day (or 0.12 μg/day with beer included) in Finland,[123] 0.114 μg/day in Spain,[106] and 0.4–3.1 μg/day in Australia.[105] Fristachi and Rice[41] estimated a daily intake of 0.11 μg/day among adults. Beer consumption makes a strong contribution to NDMA intake[105,109,123] and in our model beer consumption at the average *per capita* rate increases the dietary NDMA intake by approximately 33% in adults aged 20–49 years. Even with our estimates that were lower than those of Fristachi and Rice for dietary intake of NDMA, this source exceeds drinking water NDMA intake by a substantial margin across all age groups (Tables XIII–XV).

5.3. Role of Differing Drinking Water Factors

5.3.1. Disinfection Method (Chlorine vs. Chloramines)

As the previous survey data summarized in Table I suggested, the UCMR2 data demonstrate that NDMA is higher and more frequently detected in water systems using chloramines compared with those using free chlorine.

5.3.2. Source Waters (Surface, Groundwater, and Groundwater under the Direct Influence of Surface Water)

Given our prior understanding of factors affecting NDMA formation, notably wastewater precursors, the findings of UCMR2 are consistent in showing NDMA highest in surface waters, with next highest in groundwater under the influence of surface water, followed by mixed surface and groundwater with groundwater-only being lowest (Table XII).

5.3.3. Plant Entry Point (EP) Versus Distribution System Maximum Residence (MR) Time

Given our prior understanding that NDMA formation continues in the presence of a chloramine residual, the findings of UCMR2 with consistently higher values for MR versus EP samples were

expected (Table XII). Because consumers are served by the distribution system and we sought to avoid underestimating drinking water contribution to total human exposure, only MR samples are presented for the ADD and LADD calculations.

5.4. Our Results in Relation to Previous Published Results on POE

HC[10] and the WHO[8] considered other exogenous sources of NDMA (primarily dietary exposure) to conclude that drinking water likely comprised less than 10% of NDMA intake, but neither agency addressed endogenous exposure. Fristachi and Rice[41] considered drinking water, dietary intake, and two approaches for estimating endogenous exposure of NDMA within a Monte Carlo model. Although they did not make use of more recent dietary exposure data, nor did they have the benefit of the massive UCMR2 drinking water database for *N*-nitrosamines, the results of their model and ours are not dramatically different regarding the proportion of exogenous exposure attributed to drinking water (Table XVI, POI in our terminology).

Our results show the greatest departure from Fristachi and Rice[41] in the estimated magnitude of endogenous formation. Our lowest estimate of endogenous formation is at the level adopted by them. However, a much higher range of estimates for endogenous formation (Table IX) is supported by several studies that have a better evidence base than the lowest estimate we have.[85] We believe our comprehensive review of this topic has provided us with a more defensible basis for estimating endogenous formation of NDMA.

Fristachi and Rice[41] estimated that 0.5% of the dose was eliminated in the urine. This estimate of the fraction of a dose of NDMA that is eliminated in the urine was inappropriately drawn from individuals consuming alcohol.[78] The authors of the original paper[78] indicated that renal clearance could be no more than 0.05% in humans based on their inability to detect NDMA in subjects not consuming alcohol. Therefore, estimates of endogenous NDMA formation based upon urine elimination of NDMA should be at least 10 times that estimated by Fristachi and Rice.[41] This conclusion is supported by the low percentage of renal clearance of NDMA in animals. At a urinary elimination of 0.05%, the total daily effective dose of systemic NDMA can be calculated simply by multiplying the daily excretion rate by 2,000. In contrast, using the 0.0064% of the dose that is eliminated in the urine of the rat requires that the daily rate of excretion should be multiplied by >15,000 and the daily urine excretion of the patas monkey would be multiplied by 150,000 to arrive at the daily formation of endogenous NDMA. These large multipliers illustrate why the estimation of endogenous formation from urinary excretion rates alone is highly uncertain. However, in any of these cases, the amounts projected are orders of magnitude greater than were derived from the model system of Krul *et al.*[42] that was used by Fristachi and Rice,[41] which only considered acid-catalyzed NDMA formation. We conclude that the much larger proportion of the daily dose of NDMA arises from endogenous formation by other mechanisms.

We have detailed in Appendix B an explanation of the main differences between our approach and that of Fristachi and Rice.[41]

5.5. Implications of Results

For the U.S. EPA to develop a regulation for a drinking water contaminant, it must determine that:[124]

> (i) the contaminant may have an adverse effect on the health of persons;
>
> (ii) the contaminant is known to occur or there is a substantial likelihood that the contaminant will occur in public water systems with a frequency and at levels of public health concern; and,
>
> (iii) in the sole judgment of the Administrator, regulation of such contaminant presents a meaningful opportunity for health risk reduction for persons served by public water systems.

Because of the genotoxic carcinogenicity of *N*-nitrosamines in general, and NDMA in particular, the first of the foregoing criteria may be satisfied on the basis of additive incremental risk. The second requirement will presumably be judged by considering the UCMR2 data that are generally consistent with earlier monitoring surveys. The third requirement, to achieve "a meaningful opportunity for health risk reduction for persons served by public water systems" is much more complex because drinking water is such a negligible contributor to total human exposure to *N*-nitrosamines. The U.S. EPA has estimated that up to 100 million people may be served by water treatment systems that had at least a single detection of at least one N-nitrosamine (primarily NDMA).[125] Of course, this estimate based on any single detection is not an accurate estimate of the long-term

Table XVI. Comparison of Our Results (Based on Surface Water Treated with Chloramine, Maximum Residence, Infant Diet (Exclusively Formula-Fed) with Those of Fristachi and Rice[41]

	Fristachi and Rice[41] Tables 3 and 4			Our Model Results		
NDMA	Infant	Child	Adult	0–0.5 Years	6–12 Years	20–49 Years
Intake from drinking water	0.0013	0.0023	0.0033	0.003	0.002	0.005
Intake from diet	0.07	0.10	0.11[a]	0.04	0.05	0.06[a]
Total intake	0.07	0.10	0.11[a]	0.04	0.05	0.06[a]
POI ADD	1.9%	2.3%	3.1%	6.2%	4.0%	7.0%
POI LADD		2.8%			5.5%	
Endogenous	0	9.9	22.9–174	8–70	40–400	110–1,000 [b]
Total exposure	0.07	10.1	23.1–174	8–70	40–400	110–1,000
POE ADD	1.9%	0.02%	0.002–0.01%	0.01–0.09%	0.001–0.01%	0.001–0.01%
POE LADD		0.003–0.02%[c]			0.0002–0.001%[d] 0.0005–0.004%[e] 0.001–0.01%[f]	

Note: All values in μg/day except where noted. Where Fristachi and Rice[41] have given a range of values they correspond to endogenous estimates based on an *in vitro* model, and urinary data. Where we have given a range of values they correspond to endogenous estimates based on Dunn *et al.*[85] and Dunn *et al.*,[84] respectively. The water source type from our model for comparison is represented by the maximum NDMA category, surface water, with chloramination in the distribution system (SW/CA/MR).

[a]Exclusive of beer.

[b]Values differ from those in Table X as these are obtained using the current estimate for adult body weight from the 2011 EFH.

[c]Chlorinated and chloraminated systems and all water sources combined.

[d]Chlorinated systems using surface water and distribution system samples.

[e]Chloraminated systems using groundwater and distribution system samples.

[f]Chloraminated systems using surface water and distribution system samples, i.e., the highest category for NDMA exposure by drinking water.

exposed population. Because of the low frequency of higher levels of nitrosamines in treated drinking water, the high cost of the water analyses at ng/L levels becomes an important impact factor apart from the cost of water treatment changes. Similarly, the main disinfection source of NDMA occurrence, chloramination, has the benefit of producing lower levels of other chlorinated DBPs than does chlorination.

5.6. Study Limitations/Qualifiers

Our estimate could be improved with access to more detailed consumption data, particularly consumption of foods according to cooking method, as well as the corresponding concentration data. Cooking method has a strong influence on NDMA content, with high or direct heat being associated with higher concentrations than low or indirect methods.[126,127] In addition, our model does not take into account possible correlations between consumption of different categories of NDMA-rich foods (e.g., beer and cured pork). If these were strongly correlated then the upper bound of dietary intake would be higher, causing a lower POI for NDMA from drinking water.

The UCMR2 database is more extensive and generally better than the data for NDMA in food, but it suffers from some unfortunate deficiencies. The large proportion of responses that fail to provide any information on disinfection process used is the most serious because this lack of information makes it more difficult to assess which detailed water treatment processes contribute NDMA problems. Similarly, there is not as much clarity about how chlorine or chloramines are used by any treatment system as more detailed and complete survey reporting could have provided.

The numerical estimates of endogenous NDMA formation are uncertain, although reasonable. While the blood data provide the least ambiguous estimates of the rate of formation, it is not clear how representative these estimates are, particularly with respect to smokers versus nonsmokers. The small sample sizes and independent indications of variation among populations in the estimates based on urinary elimination suggest that the estimates may not be an

accurate reflection of the distribution of rates of formation across the whole population.

The available data on human blood concentrations and urinary excretion rates of NDMA strongly suggest that there are individuals who synthesize significant quantities of this volatile *N*-nitrosamine. However, there appear to be differences among human populations. These differences may reflect actual differences in the rates of synthesis or they may reflect differences in metabolic clearance that can be dramatically affected by exposures to other chemicals (e.g., ethanol, solvents) or from disease states (e.g., infections, diabetes). The data are not sufficiently detailed to develop a whole population distribution of values for either the blood concentrations or the amounts that are eliminated in the urine. The research describing precursors for systemic volatile *N*-nitrosation synthesis clearly indicates a potential for the formation of such products, but there are obviously intervening factors. Consequently, we could not develop a distribution of endogenous formation rates for NDMA (or other volatile *N*-nitrosamines) acceptable as an accurate description of a distribution of values representative of the whole population. Instead, we have presented a mean/95th percentile level of synthesis supported by two papers,[84,86] which represent a reasonably large number of individuals (combined $n = 105$). Despite the lower number of subjects ($n = 5$), we also present lower projections of median/mean/95th percentile derived from Dunn *et al*.[85] largely to illustrate that the likely variation within the population is much larger than suggested by the two main studies of blood concentrations from the same laboratory. We note the data from Dunn *et al*.[85] are consistent with the report of Garland *et al*.,[90] which involved a larger number of subjects ($n = 64$), but the latter detailed data were not available to us. A similar difference was noted among different studies that measured urinary excretion by identical methods. Particularly notable was that both blood concentrations and urinary excretion of NDMA was lowest in the Garland *et al.* studies.[90] The fact that similar estimates can be derived from independent studies of levels of O^6-MeG adducts in blood reinforces the fact that endogenous formation is the dominant human exposure to NDMA. Despite these difficulties, we conclude, based on all the evidence available, that endogenous formation of NDMA is orders of magnitude higher than exogenous exposure. We suspect that other volatile *N*-nitrosamine exposure would also be primarily endogenous, but there are not adequate data to make formal estimates.

6. CONCLUSIONS

The available data indicate that there is a significant rate of endogenous NDMA synthesis. Of these data, we believe the blood data to be the most dependable. However, the variation among study populations suggests that variation across the entire population is larger than suggested by the results of individual studies. Since the data are inadequate for developing a distribution for the population as a whole, we have developed two estimates of the endogenous formation from data developed by the same laboratory as an indication of how wide the variation may be in the broader population.

Using the lower estimates of endogenous NDMA formation (based on Dunn *et al.*[85]), drinking water contributes a mean LADD proportion of NDMA (POE) ranging from 0.001% for systems using free chlorine up to 0.01% for chloramination, with corresponding estimates of upper 95th percentile of 0.003% up to 0.04%. Much higher estimates of endogenous formation based on data from Dunn *et al.*[85] suggest that the POE for drinking water is much lower. About one-third of the U.S. population served by public water systems (est. 100,000,000) are exposed to any detectable NDMA from their drinking water systems.

The proportions of ADD due to drinking water are higher for infants (zero to six months) than for other age groups, largely because of the lower body weight. Using the lowest estimate for endogenous NDMA formation (based on Dunn *et al.*[85]), drinking water contributes from 0.01% to 0.09% of the ADD in exclusively formula-fed infants, for water systems using free chlorine and those using chloramination, respectively. The corresponding upper 95th percentile estimates are 0.02% and 0.3%.

From any perspective, the proportion of total NDMA exposure in humans that can be limited by controlling NDMA in drinking water is very small. Because other volatile *N*-nitrosamines are present in drinking water much less frequently and at much lower concentrations than NDMA, we would expect that if data were available the same assessment could be performed for a combination of volatile *N*-nitrosamines, but similar conclusions would likely be reached.

ACKNOWLEDGEMENTS

This research was financially supported by the American Water Works Association (AWWA) under contract to Risk Sciences International.

APPENDIX A: SENSITIVITY ANALYSIS

The proportion of exposure (POE) calculation is a straightforward ratio of *N*-nitrosodimethylamine (NDMA) exposure from drinking water to total NDMA exposure from drinking water, food, and endogenous formation. As demonstrated in the article (see Table XVI), an approximately 10-fold increase in the estimate of endogenous formation (such as occurs when the estimate is based on the data of Dunn *et al.*[84]) results in a POE lifetime average dose (LADD) value that is a 10th of the value obtained when the smaller (based on Dunn *et al.*[86]) estimate is used to represent endogenous formation.

The tornado chart (Fig. A1) illustrates the sensitivity of the POE LADD to selected inputs. In each case, the "low" value represents a value that is one-10th of the baseline level, whereas the high value represents a 10-fold increase. The length of the horizontal bars represents the divergence of the result from the baseline result, in either the positive or negative direction. Since NDMA exposure from drinking water is in the numerator of the POE LADD calculation, use of a higher estimate for this value results in a proportionally higher value for POE LADD. Conversely, the estimate for clearance of NDMA from the blood affects the denominator of POE LADD, so a higher value for clearance (and thus for blood level) is associated with a lower estimated POE LADD.

The impact of variations in dietary exposures is very small relative to the impact of endogenous formation, as dietary exposure represents only a small proportion of the denominator of the POE LADD.

The same inputs were assessed for their influence on proportion of intake (POI) LADD, as shown in Fig. A2. Clearance was omitted from Fig. A2 because endogenous exposure is not considered in the POI calculation. Again, the level of NDMA in drinking water directly affects the POI LADD, whereas individual components of dietary intake have a smaller effect. While the dietary inputs are represented here by consumption, the same effect would result if consumption values were held constant and NDMA concentration in the respective foods was allowed to vary instead.

APPENDIX B: COMPARISON BETWEEN OUR MODEL AND THAT OF FRISTACHI AND RICE

Key results of our model are compared to previously published results by Fristachi and Rice[41] in Table XVI. Whereas the estimated NDMA intake from drinking water is similar between the two sets of results, our lower estimates for dietary intake lead to a higher POI from drinking water in our model. This lower estimated dietary intake can be largely explained by lower values for NDMA concentration in foods used in our model. On the other hand, our much larger estimate of endogenous formation (based on blood levels in Dunn *et al.*[84] and Dunn *et al.*[85]) results in estimates for POE that are smaller than those calculated by Fristachi and Rice (who refer to this value as "POI, all sources") by approximately 10- to 1,000-fold. Using Dunn *et al.*[84] as the basis of the endogenous estimate, our POE for drinking water in terms of the POE LADD is 0.001% with a 95th percentile estimate of 0.005% (for water source SW/CA/MR). If our endogenous estimate is instead based on the lowest blood level reported (by Dunn *et al.*[85]), an approximately 10-fold lower blood level than that in Dunn *et al.*,[84] then our estimated drinking water POE LADD is 0.01% (95th percentile 0.04%), where again the drinking water source is represented by SW/CA/MR, and regardless of infant diet condition.

Exogenous Sources

Consumption data and body weight data for the current model were drawn from the 2011 version of the EFH[112] rather than the 1997 version. Fristachi and Rice[41] used a daily consumption of 43.6 g of dairy for a child, whereas the sum of dairy categories (whole milk, low fat or skim milk, and cheese) in our model yielded a daily consumption amount for 6–12-year olds of 13 g/kg, or about 410 g. For adults, our estimate was 280 g/day rather than the 33.6 used in Fristachi and Rice.[41]

We used narrower food categories than did Fristachi and Rice,[41] separating "dairy" into milks (whole, nonfat, and skim) and cheese, and "meats" into beef, pork, and poultry. The values for NDMA concentration in foods applied by Fristachi and Rice[41] were larger (generally by less than an order of magnitude) except for beer, in which case the value we used (from a recent survey by Baxter

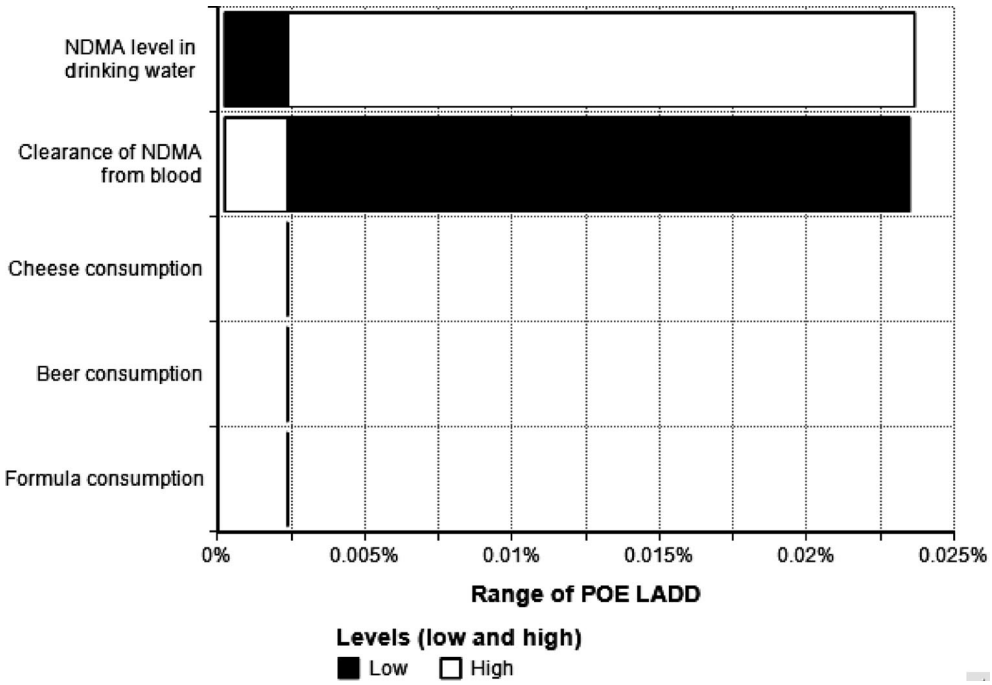

Fig. A1. Tornado chart illustrating the relative sensitivity of the POE LADD to NDMA in drinking water, endogenous formation (clearance of NDMA from blood), and major food sources.

et al.[110]), was nearly three times higher than that applied by Fristachi and Rice.

The mean level of NDMA in drinking water used in the Fristachi and Rice model (0.002 μg/L) was equal to the mean across the water source types considered in our model, derived from the UCMR2 data. Drinking water consumption volume was approximately equivalent between the two models.

Formula consumption among formula-fed infants and the level of NDMA in formula were generally consistent between our model and that of Fristachi and Rice. We assumed a drinking water component of 700 mL/day based on consumption data from the EFH 2011, in combination with a powder component equivalent to 750 mL of formula per day, based on consumption data from EFH 2011.[112] We estimated the concentration of NDMA in formula to be 0.0544 ng/mL from the powder, while the contribution from water to reconstitute was considered in the drinking water module. By comparison, Fristachi and Rice[41] assumed a formula consumption of 0.83 L/day with an NDMA level (all-inclusive) of 0.083 ng/mL. Their resulting estimate for daily NDMA intake in formula-fed infants was 0.07 μg/day, whereas we estimated an intake of 0.04 μg/day (including a contribution from small amounts of cereal) among formula-fed infants from birth to six months. In both cases, the contribution from drinking water was relatively small; 0.0013 μg/day[41] and between 0.0001 and 0.003 μg/day depending on the source (our model).

Endogenous Sources

Fristachi and Rice[41] estimated endogenous formation of NDMA in two ways, one (valued at 174 μg/day) obtained by applying an excretion rate estimate of 0.5% to urinary excretion data from Vermeer *et al.* (1998), and the other by multiplying the daily "nitrate/nitrite-rich food" consumption by a conversion factor based on Krul *et al.*[42] and yielding a mean endogenous formation of approximately 23 μg/day in adults.

We chose not to base our model on urinary levels of NDMA as the excretion rate in humans is difficult to estimate (the 0.5% value was obtained under alcohol co-administration whereas without alcohol no NDMA was recovered from human urine[78]) and in addition numerous other factors can affect levels of NDMA in the urine (see Section 4.4). Similarly, we refrained from basing our estimate on consumption of nitrate/nitrite-rich foods, both

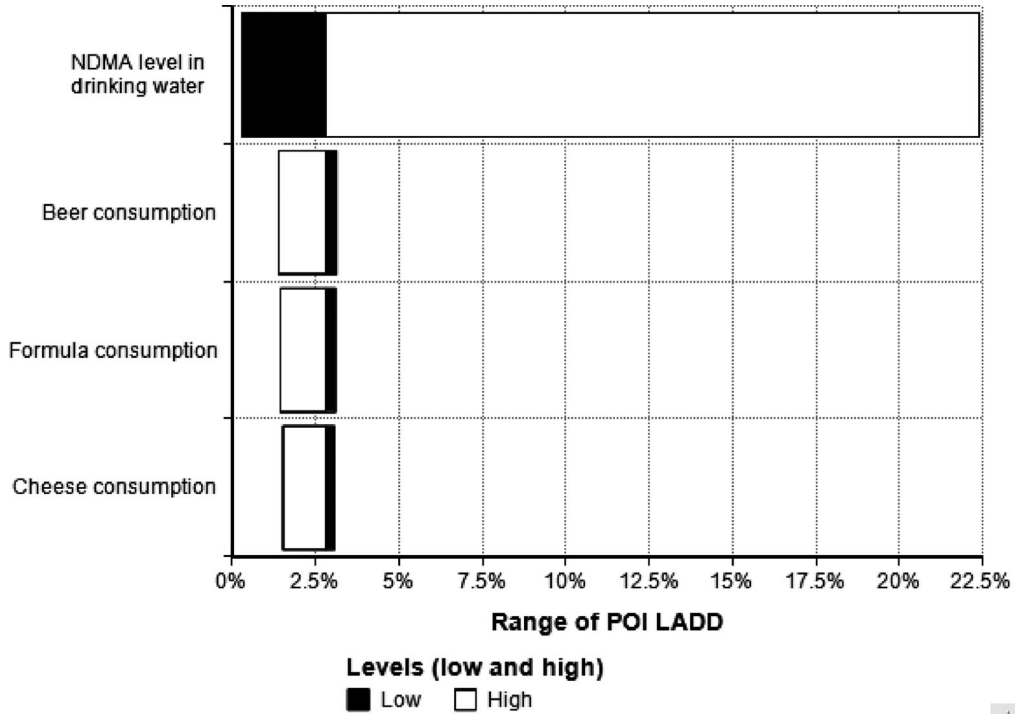

Fig. A2. Tornado chart illustrating the relative sensitivity of the POI LADD to NDMA in drinking water and major food sources.

because this method is not designed to consider non-gastric sites of formation, and because vegetables, which are the main source of nitrates in the diet, are likely to also contain inhibitors of nitrosation[128] such that the amount of NDMA formed per unit of ingested nitrate is likely to be less than that estimated by the Krul *et al.*[42] system. The relationship between stomach cancer and NDMA intake was attenuated among women with high fruit and vegetable consumption relative to those with low consumption.[129] Our estimate of endogenous formation is based instead on reported steady-state blood NDMA concentration in conjunction with a value for the rate of clearance based upon across-species scaling of animal data in the literature.

Discussion of the Fristachi and Rice[41] Assumptions

Fristachi and Rice enumerated eight assumptions underlying their model.

1. *"Individuals within a specific age group receive the same daily NDMA exposure from foods and drinking water during that exposure period."*

For each age group, our model used a Monte Carlo simulation to generate iterations in which the amount of each food and of water consumed varied according to the appropriate consumption distribution.

2. *"The bioavailability of NDMA is constant regardless of the exposure source."*

We made no specific determination of bioavailability in our model. The dominant source of NDMA is endogenous formation in systemic compartments, a source with 100% bioavailability. There is a significant effect of first-pass metabolism for orally administered (or gastrointestinally formed) NDMA in all species, but this effect is much more marked in small rodent species than in larger animals. No human-specific data on bioavailability were found, but we would assume that human bioavailablity would range from 49% (patas monkey) to 67% (swine).[70]

3. *"NDMA concentration in foods (including those calculated from non-U.S. sources) and drinking water are representative of levels encountered in the United States."*

Our model was also based on international data sources for NDMA in food because there are insufficient NDMA data in U.S. food to allow a full analysis. However, our data were limited to more

recent data to avoid using data from the early 1980s when NDMA was first raised as a concern and before actions were taken to reduce NDMA formation in many food sources. In this way, we sought to avoid overestimating the contribution of diet to total NDMA exposure.

4. *"Exposures from endogenous and exogenous exposures are additive."*

   Our model makes the same assumption.

5. *"Food intake rates follow an empirical distribution (EPA 1997a)."*

   We assumed a lognormal distribution for consumption of each food, with the exception of beer, breast milk, and formula, for which appropriate data were not available.

6. *"Water intake rates (Roseberry and Burmaster 1992) chemical concentrations (Singh et al. 1997) and body weights (Burmaster and Crouch 1997) follow a lognormal distribution."*

   In our model, body weights and drinking water consumption were assumed to be lognormal. The concentration of NDMA in foods was assumed to be a fixed value equal to the mean, which is appropriate for widely sourced, frequently consumed foods over the long term.

7. *"Bottle-fed infants do not consume foods rich in amines, nitrites, or nitrates and, consequently, do not form NDMA endogenously."*

   This assumption is based on endogenous formation of NDMA being limited to acid-catalyzed formation in the gut, which is not accurate. Because most endogenous formation occurs outside the gut, there is no reason to believe that infants do not form NDMA endogenously.

8. *"The average life expectancy in the United States is 75 years."*

   We used the same average life expectancy.

REFERENCES

1. Peto R, Gray R, Brantham P, Grasso P. Nitrosamine carcinogenesis in 5120 rodents: Chronic administration of sixteen different concentrations of NDEA, NDMA, NPYR, and NPIP in the water of 4440 inbred rats, with parallel studies on NDEA alone of the effect of age of starting (3, 6 or 20 weeks) and of species (rats, mice or hamsters). Pp. 627–665 in O'Neill IK, von Borstel RC, Miller CT, Long J, Bartsch H (eds). N-Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer (IARC Scientific Publication no. 57). Lyon, France: International Agency for Research on Cancer, 1984.
2. Peto R, Gray R, Brantham P, Grasso P. Effects on 4080 rats of chronic ingestion of *N*-nitrosodiethylamine or *N*-nitrosodimethylamine. Cancer Research, 1991; 51(23 Pt 2):6415–6451.
3. IARC (International Agency for Research on Cancer). Relevance of N-nitroso compounds to human cancer: Exposures and mechanisms. In Bartsch H (ed). Proceedings of the IXth International Symposium on N-Nitroso Compounds. New York: Oxford University Press, 1987.
4. USEPA. N-Nitrosodimethylamine (CASRN 62-75-9), Integrated Risk Information System. Available at: http://www.epa.gov/iris/subst/0045.htm, Accessed on September 14, 2012.
5. Jobb DB, Hunsinger RB, Meresz O, Taguchi VY. A study of occurrence and inhibition of formation of *N*-nitrosodimethylamine (NDMA) in the Ohsweken water supply. Pp. 103–132 in Proceedings of the American Water Works Association Water Quality Technology Conference, November 15–19, 1992, Toronto, Canada. Denver, CO, 1993.
6. Advisory Committee on Environmental Standards (ACES). A Standard for N-Nitrosodimethylamine (NDMA)—A Recommendation to the Minister of the Environment. ACES Report No. 92-01, January 1992.
7. California Department of Health Services (CDHS, now California Department of Public Health). NDMA and Other Nitrosamines—Drinking Water Issues, 2011. Available at: www.cdph.ca.gov/certlic/drinkingwater/Pages/NDMA.aspx, Accessed on September 14, 2012.
8. World Health Organization (WHO). N-Nitrosodimethylamine (NDMA), Guidelines for Drinking-Water Quality, 3rd ed. including 1st and 2nd addenda, 2008. Available at: http://www.who.int/water_sanitation_health/dwq/chemicals/ndmasummary_2ndadd.pdf, Accessed on September 14, 2012.
9. National Health and Medical Research Council (NHMRC, Australia). Australian Drinking Water Guidelines 6 2011, National Water Quality Management Strategy, 2011. Available at: http://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/eh52_aust_drinking_water_guidelines_update_120710_0.pdf, Accessed on September 14, 2012.
10. Health Canada (HC). Guidelines for Canadian Drinking Water Quality: Guideline Technical Document N-Nitrosodimethylamine (NDMA), 2011. Available at: http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/ndma/index-eng.php, Accessed on September 14, 2012.
11. USEPA. Unregulated Contaminant Monitoring Rule 2 (UCMR2), 2011. Available at: http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/ucmr2/index.cfm, Accessed on September 14, 2012.
12. Schreiber IM, Mitch WA. Nitrosamine formation pathway revisited: The importance of chloramine speciation and dissolved oxygen. Environmental Science & Technology, 2006; 40(19):6007–6014.
13. Schreiber IM, Mitch WA. Influence of the order of reagent addition on NDMA formation during chloramination. Environmental Science & Technology, 2005; 39(10):3811–3818.
14. Charrois JW, Hrudey SE. Breakpoint chlorination and free-chlorine contact time: Implications for drinking water N-nitrosodimethylamine concentrations. Water Research, 2007; 41(3):674–682.
15. Mitch WA, Sharp JO, Trussell RR, Valentine RL, Alvarez-Cohen L, Sedlak DL. N-Nitrosodimethylamine (NDMA) as a drinking water contaminant: A review. Environmental Engineering Science, 2003; 20(5):389–404.

16. Choi J, Valentine RL. Formation of *N*-nitrosodimethylamine (NDMA) from reaction of monochloramine: A new disinfection by-product. Water Research, 2002; 36(4):817–824.
17. Yang X, Fan C, Shang C, Zhao Q. Nitrogenous disinfection byproducts formation and nitrogen origin exploration during chloramination of nitrogenous organic compounds. Water Research, 2010; 44(9):2691–2702.
18. Zhou WJ, Boyd JM, Qin F, Hrudey SE, Li XF. Formation of N-nitrosodiphenylamine and two new N-containing disinfection byproducts from chloramination of water containing diphenylamine. Environmental Science & Technology, 2009; 43(21):8443–8448.
19. Najm I, Trussell RR. NDMA formation in water and waste water. Journal American Water Works Association, 2001; 93(2):92–99.
20. Kohut KD, Andrews SA. Polyelectrolyte age and N-nitrosodimethylamine formation in drinking water treatment. Water Quality Research Journal of Canada, 2003; 38(4):719–735.
21. Park SH, Wei S, Mizaikoff B, Taylor AE, Favero C, Huang CH. Degradation of amine-based water treatment polymers during chloramination as *N*-nitrosodimethylamine (NDMA) precursors. Environmental Science & Technology, 2009; 43(5):1360–1366.
22. Wilczak A, Assadi-Rad A, Lai HH, Hoover LL, Smith JF, Berger R, Rodigari F, Beland JW, Lazzelle LJ, Kinicannon EG, Baker H. Heaney CT. Formation of NDMA in chloraminated water coagulated with DADMAC cationic polymer. Journal American Water Works Association, 2003; 95(9):94–106.
23. Sacher F, Schmid, CK, Lee C, von Gunten U. Strategies for Minimizing Nitrosamine Formation during Disinfection (AwwaRF Report 91209). Denver, CO: AWWA Research Foundation, 2008.
24. Andrzejewski P, Kasprzyk-Hordern B, Nawrocki J. N-nitrosodimethylamine (NDMA) formation during ozonation of dimethylamine-containing waters. Water Research, 2008; 42(4–5):863–870.
25. Andrzejewski, P, Kasprzyk-Hordern B, Nawrocki J. The hazard of N-nitrosodimethylamine (NDMA) formation during water disinfection with strong oxidants. Desalination, 2005; 176(1–3):37–45.
26. Zhao Y-Y, Boyd JM, Woodbeck M, Andrews RC, Qin F, Hrudey SE, Li X-F. Formation of *N*-nitrosamines from eleven disinfection treatments of seven different surface waters. Environmental Science & Technology, 2008; 42(13):4857–4862.
27. Krasner SW. The formation and control of emerging disinfection by-products of health concern. Philosophical Transactions of the Royal Society A, 2009; 367(1904):4077–4095.
28. Stefan MI, Bolton JR. UV direct photolysis of *N*-nitrosodimethylamine (NDMA): Kinetic and product study. Helvetica Chimica Acta, 2002; 85:1416–1426.
29. Sharpless CM, Linden KG. Experimental and model comparisons of low- and medium-pressure Hg lamps for the direct and H_2O_2 assisted UV photodegradation of *N*-nitrosodimethylamine in simulated drinking water. Environmental Science & Technology, 2003; 37(9):1923–1940.
30. Krasner SW. Halogenated DBPs and emerging issues. Pp. 59–71 in Hrudey SE & Charrois JWA (eds). Disinfection By-Products and Human Health. London: IWA Publishing, 2012.
31. Charrois JW, Arend MW, Froese KL, Hrudey SE. Detecting N-nitrosamines in drinking water at ng/L levels using ammonia positive chemical ionisation. Environmental Science & Technology, 2004; 38(18):4835–4841.
32. Mitch WA, Sedlak DL. Factors controlling nitrosamine formation during wastewater chlorination. Water Science & Technology: Water Supply, 2002; 2(3):191–198.
33. Zhao YY, Boyd JM, Hrudey SE, Li XF. Characterization of new nitrosamines in drinking water using liquid chromatography tandem mass spectrometry. Environmental Science & Technology, 2006; 40(24):7636–7641.
34. Barrett S, Hwang C, Guo Y, Andrews SA, Valentine R. Occurrence of NDMA in drinking water: A North American Survey, 2001–2002. In Proceedings, American Water Works Association Annual Conference and Exhibition, Anaheim, CA, June 15, 2003.
35. Valentine RL, Barrett SE, Andrews SA, Fitzsimmons S. Factors Affecting the Formation of NDMA in Water and Occurrence. Project #2678, Denver, CO: AWWA Research Foundation, 2005.
36. Charrois JW, Boyd JM, Froese KL, Hrudey SE. Occurrence of N-nitrosamines in Alberta public drinking water distribution systems. Journal of Environmental Engineering and Science, 2007; 6(1):103–114.
37. Mitch WA, Krasner SW, Westerhoff P, Dotson A. Occurrence and Formation of Nitrogenous Disinfection By-Products. Denver, CO: Water Research Foundation, 2009.
38. Goslan EH, Krasner SW, Bower M, Rocks SA, Holmes P, Levy LS, Parsons SA. A comparison of disinfection by-products found in chlorinated and chloraminated drinking waters in Scotland. Water Research, 2009; 43(18):4698–4706.
39. Dillon G, Blake S, Rumsby P, Rockett L, Hall T, Jackson P, Rawlinson A. NDMA: Concentrations in Drinking Water and Factors Affecting Its Formation, Final Report (Report No. 7348. Contract No. 14636-0). London: DEFRA, 2008.
40. Asami M, Oya M, Kosaka K. A nationwide survey of NDMA in raw and drinking water in Japan. Science of the Total Environment, 2009; 407(11):3540–3545.
41. Fristachi A, Rice G. Estimation of the total daily oral intake of NDMA attributable to drinking water. Journal of Water and Health, 2007; 5(3):341–355.
42. Krul CA, Zeilmaker MJ, Schothorst RC, Havenaar R. Intragastric formation and modulation of N-nitrosodimethylamine in a dynamic in vitro gastrointestinal model under human physiological conditions. Food and Chemical Toxicology, 2004; 42(1):51–63.
43. Tricker AR, Pfundstein B, Kälble T, Preussmann R. Secondary amine precursors to nitrosamines in human saliva, gastric juice, blood, urine, and faeces. Carcinogenesis, 1992; 13(4):563–568.
44. Mirvish SS. Formation of *N*-nitroso compounds: Chemistry, kinetics, and *in vivo* occurrence. Toxicology and Applied Pharmacology, 1975; 31(3):325–351.
45. Ohshima H, Mahon GA, Wahrendorf J, Bartsh H. Dose-response study of N-nitrosoproline formation in rats and a deduced kinetic model for predicting carcinogenic effects caused by endogenous nitrosation. Cancer Research, 1983; 43(11):5072–5076.
46. Bryan NS, Rassaf T, Maloney RE, Rodriguez CM, Saijo F, Rodriguez JR, Feelisch M. Cellular targets and mechanisms of nitros(yl)ation: An insight into their nature and kinetics in vivo. Proceedings of the National Academy of Sciences (USA), 2004; 101(12):4308–4313.
47. Wu Y, Brouet I, Calmels S, Bartsch H, Ohshima H. Increased endogenous N-nitrosamine and nitrate formation by induction of nitric oxide synthase in rats with acute hepatic injury caused by *Propionibacterium acnes* and lipopolysaccharide administration. Carcinogenesis, 1993; 14(1):7–10.
48. Lijinsky W, Reuber MD. Transnitrosation by Nitrosamines In Vivo, Pp. 625–638, (IARC Scientific Publication no. 41) 1982.
49. Cross AJ, Pollock JR, Bingham SA. Haem, not protein or inorganic iron, is responsible for endogenous intestinal *N*-nitrosation arising from red meat. Cancer Research, 2003; 63(10):2358–2360.

50. Lunn JC, Kuhnle G, Mai V, Frankenfeld C, Shuker DE, Glen RC, Goodman JM, Pollock JR, Bingham SA. The effect of haem in red and processed meat on the endogenous formation of *N*-nitroso compounds in the upper gastrointestinal tract. Carcinogenesis, 2007; 28(1):685–690.
51. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell, 2010; 140(6):883–899.
52. Leaf CD, Wishnok JS, Tannenbaum SR. Endogenous incorporation of nitric oxide from L-arginine into *N*-nitrosmorpholine stimulated by *Escherichia coli* lipopolysaccharide in the rat. Carcinogenesis, 1991; 12(3):537–539.
53. Grisham MB, Ware K, Gilleland HE Jr, Gilleland LB, Abell CL, Yamada T. Neutrophil-mediated nitrosamine formation: Role of nitric oxide in rats. Gastroenterology, 1992; 103:1260–1266.
54. Liu, RH, Baldwin B, Tennant BC, Hotchkiss JH. Elevated formation of nitrate and N-nitrosodimethylamine in woodchucks (*Marmota monax*) associated with chronic woodchuck hepatitis virus infection. Cancer Research, 1991; 51:3925–3929.
55. Liu, RH, Jacob JR, Tennant BC, Hotchkiss JH. Nitrite and nitrosamine synthesis by hepatocytes isolated from normal woodchucks (*Marmota Monax*) and woodchucks chronically infected with woodchuck hepatitis virus. Cancer Research, 1992; 52:4139–4143.
56. Ohshima H, Bandaletova TY, Brouet I, Bartsch H, Kirby G, Ogunbiyi F, Vatanasapat V, Pipitgool V. Increased nitrosamine and nitrate biosynthesis mediated by nitric oxide synthase induced in hamsters infected with liver fluke (*Opisthorchis viverrini*). Carcinogenesis, 1994; 15(2):271–275.
57. Satarug S, Haswell-Elkins MR, Tsuda M, Mairiang P, Sithithaworn P, Mairiang E, Esumi H, Sukprasert S, Yongvanit P, Elkins DB. Thiocyanate-independent nitrosation in humans with carcinogenic parasite infection. Carcinogenesis, 1996; 17(5):1075–1081.
58. Tricker AR, Mostafa MH, Spiegelhalder B. *N*-nitroso compounds in *Schistosomaiasis* and bilharzia bladder cancer patients. Carcinogenesis, 1989; 10(3):547–552.
59. Stickler DJ, Phil D, Chawla JC, Tricker AR, Preussman R. N-nitrosamine generation by urinary tract infections in spine-injured patients. Paraplegia, 1992; 30:855–863.
60. Hoehn T, Stiller B, McPhaden AR, Wadsworth RM. Nitric oxide synthases in infants and children with pulmonary hypertension and congenital heart disease. Respiratory Research, 2009; 10:110–118.
61. Dixon JS, Jen PY, Gosling JA. Immunohistochemical characteristics of human paraganglion cells and sensory corpuscles associated with the urinary bladder. A developmental study in the male fetus, neonate and infant. Journal of Anatomy, 1998; 192(Pt 3):407–415.
62. Ratliff B, Sekulic M, Rodebaugh J, Solhaug MJ. Angiotensin II regulates nitric oxide synthase expression in afferent arterioles of the developing porcine kidney. Pediatric Research, 2010; 68(1):29–34.
63. Mata-Greenwood E, Jenkins C, Farrow KN, Konduri GG, Russell JA, Lakshminrusimha S, Black SM, Steinborn RH. eNOS function is developmentally regulated: Uncoupling of eNOS occurs postnatally. American Journal of Physiology – Lung Cellular and Molecular Physiology, 2006; 290(2):L232–L241.
64. Monau TR, Vargus VE, King N, Yellon SM, Myers DA, Ducsay CA. Long-term hypoxia increases endothelial nitric oxide synthase expression in the ovine fetal adrenal. Reproductive Sciences, 2009; 16(9):865–874.
65. Streeter AJ, Nims RW, Sheffels PR, Heur YH, Yang CS, Mico BA, Gombar CT, Keefer LK. Metabolic denitrosation of *N*-nitrosodimethylamine *in vivo* in the rat. Cancer Research, 1990; 50(4):1144–1150.
66. Keefer LK, Anjo T, Wade D, Wang T, Yang CY. Concurrent generation of methylamine and nitrite during denitrosation of N-nitrosodimethylamine by rat liver microsomes. Cancer Research, 1987; 47(2):447–452.
67. Lee VM, Keefer LK, Archer MC. An evaluation of the roles of metabolic denitrosation and α-hydroxylation in the hepatotoxicity of N-nitrosodimethylamine. Chemical Research in Toxicology, 1996; 9(8):1319–1324.
68. Hecht SS, Young R. Metabolic α-hydroxylation of *N*-nitrosomorpholine and 3,3,5,5-tetradeutero-N-nitrosmorpholine in the F344 rat. Cancer Research, 1981; 41(12 Pt 1):5039–5043.
69. Yoo JS, Ishizaki H, Yang CS. Roles of cytochrome P450IIE1 in the dealkylation and dinitrosation of *N*-nitrosodimethylamine and *N*-nitrosodiethylamine in rat liver microsomes. Carcinogenesis, 1990; 11(12):2239–2243.
70. Gombar CT, Harrington GW, Pylypiw HM Jr, Anderson LM, Palmer AE, Rice JM, Magee PN, Burak ES. Interspecies scaling of the pharmacokinetics of *N*-nitrosodimethylamine. Cancer Research, 1990; 50(14):4366–4370.
71. Borlakoglu JT, Scott A, Henderson CJ, Wolf CR. Expression of P450 isoenzymes during rat liver organogenesis. International Journal of Biochemistry, 1993; 25(11):1659–1668.
72. Carpenter SP, Savage DD, Schultz ED, Raucy JL. Ethanol-mediated transplacental induction of CYP2E1 in fetal rat liver. Journal of Pharmacology and Experimental Therapeutics, 1997; 282(2):1028–1036.
73. Yoo J-S, Ning SM, Patten CJ, Yang CS. Metabolism and activation of *N*-nitrosodimethylamine by hamster and rat microsomes: Comparative study with weanling and adult animals. Cancer Research, 1987; 47(4):992–998.
74. Blanco JG, Harrison PL, Evans WE, Relling MV. Human cytochrome P450 maximal activities in pediatric versus adult liver. Drug Metabolism and Disposition, 2000; 28(4):379–382.
75. Kraft PL, Skipper PL, Charmley G, Tannenbaum SR. Urinary excretion of dimethylnitrosamine: A quantitative relationship between dose and urinary excretion. Carcinogenesis, 1981; 2(7):609–612.
76. Anderson LM, Koseniauskas R, Burak ES, Moskal TJ, Gombar CT, Phillips JM, Sansone EB, Kemig S, Magee PN, Rice JM. Reduced blood clearance and increased urinary excretion of N-nitrosodimethylamine in patas monkeys exposed to ethanol or isopropyl alcohol. Cancer Research, 1992; 52(6):1463–1468.
77. Gombar CT, Plylypiw HM Jr, Harrington GW. Pharmacokinetics of N-nitrosodimethylamine in beagles. Cancer Research, 1987; 47(2):343–347.
78. Spiegelhalder B, Eisenbrand G, Preussmann R. Urinary excretion of N-nitrosamines in rats and humans. Pp. 443–449 in O'Neill IK, von Borstel RC, Miller CT, Long J, Bartsch H, eds. N-Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer (IARC Scientific Publications, No. 57). Lyon, France: International Agency for Research on Cancer, 1984.
79. Fine DH, Challis BC, Hartman P, Van Ryzin J. Endogenous Synthesis of Volatile Nitrosamines: Model Calculations and Risk Assessment. Pp. 379–396 in Bartsch H, O'Neill IK, Castegnaro M, Okada M (eds). N-Nitroso Compounds: Occurrence and Biological Effects (IARC Scientific Publications No. 41). Lyon, France: International Agency for Research on Cancer, 1982.
80. Fine DH, Ross R, Roundbehler DP, Silvergeld A, Song L. Formation *in vivo* volatile *N*-nitrosamines in man after ingestion of cooked bacon and spinach. Nature, 1977; 265:753–755.
81. Gough TA, Webb KS, Swann PF. An examination of human blood for the presence of volatile nitrosamines. Food and Chemical Toxicology, 1983; 21(2):151–156.

82. Lakritz L, Pensabene JW. Survey of human milk for volatile N-nitrosamines and the influence of diet on their formation. Food and Chemical Toxicology, 1984; 22(9):721–724.
83. Lakritz L, Simenhoff ML, Dunn SR, Fiddler W. N-Nitrosodimethylamine in human blood. Food and Cosmetics Toxicology, 1980; 18(1):77–79.
84. Dunn SR, Pensabene JW, Simenhoff ML. Analysis of human blood for volatile N-nitrosamines by gas chromatography-chemiluminescence detection. Journal of Chromatography, 1986; 377:35–47.
85. Dunn SR, Simenhoff ML, Lele PS, Goyal S, Pensabene JW, Fiddler W. N-nitrosodimethylamine blood levels in patients with chronic renal failure: Modulation of levels by ethanol and ascorbic acid. Journal of the National Cancer Institute, 1990; 82(9):783–787.
86. Simenhoff ML, Dunn SR, Kirkwood RG, Fiddler W, Pensabene JW. Presence of nitrosamines in blood of normal and diseased human subjects. Pp. 283–294 in Magee PN (ed). Nitrosamines and Human Cancer. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1982 (Banbury Report 12).
87. Lele PS, Dunn SR, Simenhoff ML, Fiddler W, Pensabene JW. Evidence for generation of the precarcinogen nitrosodimethylamine in the small intestine in chronic renal failure. Kidney International Supplement, 1983; 16:S229–233. (Abstract only.)
88. Simenhoff ML, Dunn SR, Lele PS. Analysis for and intestinal metabolism of precursor nitroso compounds in normal subjects and in patients with chronic renal failure. Pp. 161–170 in O'Neill IK, von Borstel RC, Miller CT, Long J, Bartsch H (eds). N-Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer (IARC Scientific Publications No. 57). Lyon, France, International Agency for Research on Cancer, 1984.
89. Simenhoff ML, Dunn SR. Altered gut flora in uremia. Journal of Renal Nutrition, 1996; 6(2):68–74.
90. Garland WA, Kuenzig W, Rubio F, Kornychuk H, Norkus EP, Conney AH. Urinary excretion of nitrosodimethylamine and nitrosoproline in humans: Interindividual and intraindividual differences and the effect of administered ascorbic acid and α-tocopherol. Cancer Research, 1986; 46(10):5392–5400.
91. van Maanen JM, Welle IJ, Hageman G, Dallinga JW, Mertens PL, Kleinjans JC. Nitrate contamination of drinking water: Relationship with HPRT variant frequency in lymphocyte DNA and urinary excretion of *N*-nitrosamines. Environmental Health Perspectives, 1996; 104(5):522–528.
92. van Maanen JM, Pachen DM, Eng M, Dallinga JW, Kleinjans JC. Formation of nitrosamines during consumption of nitrate- and amine-rich foods, and the influence of the use of mouthwashes. Cancer Detection and Prevention Journal, 1998; 22(3):204–212.
93. Vermeer IT, Pachen DM, Dallinga JW, Kleinjans JC, van Maanen JM. Volatile *N*-nitrosamine formation after intake of nitrate and the ADI level in combination with an amine-rich diet. Environmental Health Perspectives, 1998; 106(8):459–463.
94. Vermeer IT, Moonen EJ, Dallinga JW, Kleinjans JC, van Maanen JM. Effect of ascorbic acid and green tea on endogenous formation of N-nitrosodimethylamine and N-nitrosoproline in humans. Mutation Research, 1999; 428(1–2):353–361.
95. Levallois P, Ayotte P, van Maanen JM, DeRosiers T, Gingras S, Dallinga JW, Vermeer IT, Zee J, Poirier G. Excretion of volatile nitrosamines in a rural population in relation to food and drinking water consumption. Food and Chemical Toxicology, 2000; 38(11):1013–1019.
96. Farmer PB, Shuker DE. What is the significance of increases in background levels of carcinogen-derived protein and DNA adducts? Some considerations for incremental risk assessment. Mutation Research, 1999; 424(1–2):275–286.
97. Margison GP, Povey AC, Kaina B, Koref MS. Variability and regulation of O^6-alkylguanine-DNA alkyltransferase. Carcinogenesis, 2003; 24(4):625–635.
98. Kyrtopoulos SA. DNA adducts in humans after exposure to methylating agents. Mutation Research, 1998; 405(2):135–143.
99. Georgiadis P, Samoli E, Kaila S, Katsouyanni K, Krytopoulos SA. Ubiquitous presence of O^6-methylguanine in human peripheral blood and cord blood DNA. Cancer Epidemiology, Biomarkers and Prevention, 2000; 9:299–305.
100. Georgiadis P, Kaila S, Makedonopoulou P, Fthenou E, Chatzi L, Pietsa V, Kyrtopoulos SA. Development and validation of a new, sensitive immunochemical assay for O^6-methylguanine in DNA and its application in a population study. Cancer Epidemiology, Biomarkers and Prevention, 2010; 20(1):82–90.
101. Cuny G, Soriano P, Macaya G, Bernardi G. The major components of the mouse and human genomes. I. Preparation, basic properties, and compositional heterogeneity. European Journal of Biochemistry, 1981; 115(2):227–233.
102. Chhabra SK, Souliotis V L, Harbaugh JW, Krasnow SW, Jones AB, Anderson LM, Krytopoulos SA. O^6-Methylguanine DNA adduct formation and modulation by ethanol in placenta and fetal tissues after exposure of pregnant patas monkeys to *N*-nitrosodimethylamine. Cancer Research, 1995; 55(24):6017–6020.
103. Souliotis VL, Henneman JR, Reed CD, Chhabra SK, Diwan BA, Anderson LM, Krytopoulos SA. DNA adducts and liver DNA replication in rats during chronic exposure to N-nitrosodimethylamine (NDMA) and their relationships to the dose-dependence of NDMA hepatocarcinogenesis. Mutation Research, 2002; 500(1–2):75–87.
104. Souliotis VL, Chhabra S, Anderson LM, Krytopoulos SA. Dosimetry of O^6-methylguanine in rat DNA after low-dose, chronic exposure to N-nitrosodimethylamine (NDMA). Implications for the mechanism of NDMA hepatocarcinogenesis. Carcinogenesis, 1995; 16(10):2381–2387.
105. Schäfer AI, Mitch W, Walewijk S, Munoz A, Teuten E, Reinhard M. Micropollutants in water recycling: A case study of N-nitrosodimethylamine (NDMA) exposure from water versus food. Pp. 203–228 in Escobar IC, Schäfer AI (eds). Sustainability Science and Engineering, Volume 2. Amsterdam: Elsevier B.V., 2010.
106. Jaksyzn P, Ibàñez R, Pera G, Agudo A, Carcia-Closas R, Amiano P, Gonzàlez CA. Food Content of Potential Carcinogens. Barcelona: Catalan Institute of Oncology, 2004.
107. Stuff JE, Goh ET, Barrera SL, Bondy ML, Forman MR. Construction of an N-nitroso database for assessing dietary intake. Journal of Food Composition and Analysis, 2009; 22S:S42–S47.
108. Griesenbeck JS, Steck MD, Huber JC, Sharkey JR, Rene AA, Brender JD. Development of estimates of dietary nitrates, nitrites, and nitrosamines for use with the short willet food frequency questionnaire. Nutrition Journal, 2009; 8:16–25.
109. Biaudet H, Mavelle T, Debry G. Mean daily intake of N-nitrosodimethylamine from foods and beverages in France in 1987–1992. Food and Chemical Toxicology, 1994; 32(5):417–421.
110. Baxter ED, Slaiding IR, Travers V. Current incidence of N-nitrosodimethylamine in beers worldwide. Food Additives & Contaminants: Part A, 2007; 24(8):807–811.
111. FDA. Total Diet Study Version 3. 2001. Available at: http://www.fda.gov/Food/FoodSafety/FoodContaminants

Adulteration/TotalDietStudy/default.htm, Accessed on September 17, 2012.
112. USEPA. Exposure Factors Handbook 2011 Edition (Final), 2011. http://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252, Accessed on September 17, 2012.
113. Uibu J, Tauts O, Levin A, Shimanovskaya N, Matto R. N-nitrosodimethylamine, nitrate and nitrate-reducing microorganisms in human milk. Acta Paediatrica, 1996; 85(10):1140–1142.
114. Jurado-Sànchez B, Ballesteros E, Gallego M. Gas chromatographic determination of N-nitrosamines, aromatic amines, and melamine in milk and dairy products using an automatic solid-phase extraction system. Journal of Agricultural and Food Chemistry, 2011; 59(13):7519–7526.
115. Grummer-Strawn LM, Scanlon KS, Fein SB. Infant feeding and feeding transitions during the first year of life. Pediatrics, 2008; 122(2):S36–S42.
116. Joeckel RJ, Phillips SK. Overview of infant and pediatric formulas. Nutrition in Clinical Practice, 2009; 24(3):356–362.
117. Weston RJ. Trace amounts of nitrosamines in powdered milk and milk proteins. Journal of the Science of Food and Agriculture, 1983; 34(8):893–895.
118. Havery DC, Hotchkiss JH, Fazio T. Rapid determination of volatile N-nitrosamines in nonfat dry milk. Journal of Dairy Science, 1982; 65(2):182–185.
119. Libbey LM, Scanlan RA, Barbour JF. N-nitrosodimethylamine in dried dairy products. Food and Cosmetics Toxicology, 1980; 18(5):459–461.
120. EPA. Exposure Factors Handbook (1997 Final Report), 1997. Available at: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=12464&CFID=99360737&CFTOKEN=72633444&jsessionid=4e30aaff81c61b290cf5342a42266e6568b2, Accessed on August 26, 2012.
121. Tannenbaum SR. A model for estimation of human exposure to endogenous N-nitrosodimethylamine. Oncology, 1980; 37(4):232–235.
122. Tricker AR, Pfundstein B, Theobald E, Preussmann R, Spiegelhalder B. Mean daily intake of volatile N-nitrosamines from foods and beverages in West Germany in 1989–1990. Food and Chemical Toxicology, 1991; 29(11):729–732.
123. Dich J, Järvinen R, Knekt P, Penttilä P-L. Dietary intakes of nitrate, nitrite and NDMA in the Finnish Mobile Clinic Health Examination Survey. Food Additives and Contaminants, 1996; 13(5):541–552.
124. USEPA. Paradigm for Addressing Drinking Water Contaminants as Groups to Enhance Public Health Protection. EPA Draft Discussion Paper, July 27, 2010.
125. USEPA. Regulatory Determinations for the Third Drinking Water Contaminant Candidate List Stakeholder Meeting. Washington DC, June 16, 2011.
126. Miller BJ, Billadeau SM, Miller DW. Formation of N-nitrosamines in microwaved versus skillet-fried bacon containing nitrite. Food and Chemical Toxicology, 1989; 27(5):295–299.
127. Lee SJ, Shin JH, Sung NJ, Kim JG, Hotchkiss JH. Effect of cooking on the formation of N-nitrosamine in Korean dried seafood products. Food Additives and Contaminants, 2003; 20(1):31–36.
128. Bartsch H, Ohshima H, Pignatelli B. Inhibitors of endogenous nitrosation. Mechanisms and implications in human cancer prevention. Mutation Research, 1988; 202(2):307–324.
129. Larsson SC, Bergkvist L, Wolk A. Processed meat consumption, dietary nitrosamines and stomach cancer risk in a cohort of Swedish women. International Journal of Cancer, 2006; 119(4):915–919.